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Impacts of fluoride contamination on food crops grown in rural areas of the African Rift Valley

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Summary

The intake of fluoride-rich food products can represent a major source of fluoride exposure for human organisms, contributing, together with the consumption of contaminated drinking-water, to enhance the hazard of incurring severe diseases. Endemic fluorosis, in fact, is a major illness caused by the chronic exposure to fluoride, which affects the dental apparatus and the skeletal system but also, as recently observed, the internal organs, and the nervous, reproductive and immune systems. Even though the connection between the environmental fluoride contamination and the fluorosis disease is well recognised, the information on the uptake behaviour and the effects of fluoride on crops is still limited and fragmented, particularly for some strongly affected areas, such as the East African Rift Valley, where over 90% of the population was observed to suffer symptoms of fluorosis. With the aim to provide a comprehensive scientific overview on this topic, the first chapter of this thesis, attempted to gather all the existing literature data to review, through a systematic approach, the current state of knowledge on the impacts of the environmental fluoride pollution on crop productivity and safety. The second chapter is focused on the outcomes of a field-scale study implemented in three case study areas of the Ngarenanyuki region, in Arumeru District, Arusha, North Tanzania, with the objective to evaluate the fluoride uptake and partitioning between edible (leaves, fruits or seeds) and not edible portions of the main cultivated and consumed crops of the area. Considering the dietary habits of the endemic population, the outcomes of the study suggest that the investigated crops can substantially contribute to fluoride related diseases, especially in earlier ages. In the third chapter, three of the crops tested in the field (maize, bean and tomato) were also investigated under controlled conditions at mesocosm-scale in order to better understand the impacts of increasing fluoride concentration in the irrigation water on the productivity and the fluoride accumulation pattern of the studied species. Among the investigated crops, the greater tolerance to fluoride was observed in maize and bean. Fluoride concentration in edible parts of all the three crops was not influenced by the increasing levels of fluoride in the irrigation water, while in crop residues the accumulation was fluoride dose-dependent, representing an indirect risk for human health. In fact, in the study area crop residues of maize and bean are mainly used for feeding livestock so that fluoride may enter the food chain through the consumption of animal products.

Chapter I

Impacts of soil and water fluoride contamination on the safety and productivity of food and feed crops: A systematic review

Impacts of soil and water fluoride contamination on the safety and productivity of food and feed crops: A systematic review

Abstract

Although a strong connection between the environmental fluoride contamination and the fluorosis disease is nowadays worldwide well documented, the knowledge of the fluoride contamination levels of cultivated crops at the basis of the human food-chain is limited and fragmented. Adopting a systematic approach, this study reviews the available literature concerning the impacts of soil and water fluoride pollution on the safety and productivity of food and feed crops at a global scale, with the aim to provide a comprehensive overview of the current state of the art. The analyses of literature highlighted that food and feed crops exposed to soil and water fluoride pollution may reach concentrations of fluoride potentially harmful for human health. Nevertheless, despite the efforts already made to assess crop fluoride accumulation in contaminated areas of India and China, the present study brings to light the lack of knowledge still existing on this issue for some regions strongly affected by environmental fluoride contamination such as the East African Rift Valley.

Concerning the impacts of fluoride on cultivated crops, many authors observed that fluoride can produce toxic effects on plants such as oxidative stress, reduction in chlorophyll content, alterations in the level of glutathione, ascorbic acid, total flavonoid, total polyphenol and macro and micronutrients. However, the appearance of symptoms such as visible injuries, reduced root and shoot length and yield decline were not always observed, also at high levels of fluoride exposure, and in some cases the biomass production was even stimulated by increasing fluoride levels.

Keywords: Fluoride, water pollution, soil pollution, crops, food safety.

Introduction

The connection between excessive environmental fluoride occurrence and high rate of fluorosis in the populations of polluted areas is nowadays well recognized (Ozsvath, 2009). The prolonged exposure to high fluoride doses can lead, in fact, to a series of major illnesses that involve the dental apparatus and skeletal system but also, as recently examined, non-skeletal tissues as internal organs, nervous, reproductive and immune systems (Wei, et al., 2019). Although low-fluoride intakes have been recognised to have positive effects on the prevention of dental caries, moderate chronic ingestion, particularly during childhood, is known to provoke the appearance of black spots on teeth and in most severe cases their loss. Skeletal system disorders appear with the protracted intake of higher fluoride doses with symptoms that comprise bone deformities, ligaments calcification and associated joint, back, knee, shoulder and neck pain with difficulties in movements and in proper walking (Bharati, et al., 2005, WHO, 1999, 2011).

Endemic fluorosis is prevalent in at least 25 countries with the highest incidence in India, China and various African regions (Vithanage and Bhattacharya, 2015), but groundwater with concentrations above the WHO guideline limit for drinking-water (1.5 mg L^{-1}) have been found in many parts of the world. Beyond China and India, affected regions are Sri Lanka, North and West African countries (Tunisia, Libya, Sudan, Senegal, Ghana, Ivory Coast), the East African Rift Valley (Kenya, Tanzania, Ethiopia, Uganda and Rwanda), South Africa, central Argentina, northern Mexico and Pakistan (Edmunds and Smedley, 2013).

The sources of fluoride contamination can be both natural and anthropogenic with the firsts being paramount (Amini, et al., 2008). Natural sources are related to geogenic processes that are primarily responsible for groundwater pollution, such as for example the weathering of F-rich minerals and the release of fluoride element due to volcanic activities, fumarolic gases, hydro-geothermal vents and marine aerosols. Contamination from anthropogenic activities can be significant on a local scale and may be either of industrial origin, such as the release of wastes from aluminium smelters, effluents

from coal-based power stations and factories processing various materials (e.g glass, ceramic, plastics, pesticides, disinfectants etc.), or agricultural-related as for example the long-term uncontrolled use of phosphatic fertilizer or the irrigation with fluoride-enriched water (Ali, et al., 2016, Kimambo, et al., 2019, Singh, et al., 2018)

Through the polluted soil, water, and air, fluoride can enter in the human food-chain (Vithanage and Bhattacharya, 2015). After drinking water, in fact, the consumption of contaminated food products is considered another important source of fluoride exposure for human organisms (Brahman, et al., 2014, WHO, 2011).

Several field-scale explorative trials and controlled experiments have been conducted in various contaminated regions of the world to investigate the accumulation of fluoride in crops and the effects on their productivity, nevertheless, the information is still fragmented.

Even though, in fact, numerous studies have considered and examined the worldwide environmental fluoride occurrence, particularly in groundwater systems (Amini, et al., 2008, Edmunds and Smedley, 2013, Kimambo, et al., 2019, Vithanage and Bhattacharya, 2015), and various overviews have been conducted on fluoride toxicity in plants (Baunthiyal and Ranghar, 2014, Baunthiyal and Ranghar, 2015, Choudhary, et al., 2019, Singh, et al., 2018, Yadu, et al., 2016), no systematic reviews have been performed concerning the impacts of soil and water fluoride pollution on the safety and productivity of food and feed crops at global scale. Considered all the above, with the aim to provide a comprehensive information on this topic and bring to light the gaps of knowledge that still need to be filled, this work attempted to gather all the existing literature data to review the current state of knowledge on the impacts of the environmental fluoride pollution on crop productivity and safety.

Therefore, this review can provide a comprehensive scientific overview to scientist and policymakers in order to better support decision making concerning further investigation trends and effective actions toward the development and implementation of possible mitigation strategies in the affected areas.

Review methods

The systematic literature review process followed the methodological approach reported by existing guidelines (Bilotta, et al., 2014, Collaboration for Environmental Evidence, 2013, Pullin and Stewart, 2006, Pullin and Stewart, 2009). A review protocol was implemented defining the connotations of the systematic literature search, the inclusion/exclusion criteria and the data extraction forms (Annex 1). Academic search engines such as ISI Web of Science (Web of Science Core Collection plus other databases such as Bioabs, Kjd, Medline, Rsci, Scielo), PubMed, Scopus, Science Direct, JSTOR, Agricola, Fluorideresearch.org and Cab Abstracts were used. Advanced search options were considered entering the following keywords: *fluoride OR fluorine*, in the title field, *AND crop OR plant OR vegetation OR grasses OR vegetables OR agriculture*, in the others fields. The search was restricted to the English language, and the timespan went from 1985 to 2020. To refine the scoping results the search conducted on the Web of Science Core Collection and the Scopus database was limited to the following subject areas:

- for the Web of Science Core Collection: *Environmental sciences, Toxicology, Water resources, Plant sciences, Soil science, Agronomy, Ecology, Agriculture multidisciplinary, Multidisciplinary sciences, Biology, Forestry, Horticulture Agricultural engineering, Physiology, Agriculture dairy animal science, Environmental studies*
- for the Scopus database: *Environmental Science, Agricultural and Biological Sciences, Multidisciplinary, Decision Sciences*

Records were stored in a reference manager software database and duplicates were eliminated. The article screening was performed following a stepwise process according to the established inclusion criteria (Table 1). A first selection was conducted on the basis of the publication title, in the second stage abstracts were assessed and finally, the remaining articles were filtered revising the whole paper. In case of doubts about the pertinence of a record, this was included and further evaluated in the next stage. A subset of the references was revised by two further independent reviewers in order

to assess the reliability of the application of the inclusion criteria. The final numbers of articles recovered, duplicated, accepted and excluded were annotated to produce an accurate flow diagram (Annex 2). The list of the 85 papers that were finally included is reported in Annex 3.

Table 1 - Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Every kind of study assessing: ▪ the effects of soil and water fluoride on quali-quantitative characteristics of crop production ▪ fluorine uptake capacity of different kind of crops cultivated in soils and/or irrigated with water characterized by various levels of fluoride ▪ articles focusing only on adults plants ▪ microcosm, mesocosm and field-scale trials	First and second stages (screening title and abstract): ▪ Not on topic (misclassified) ▪ No relevant population (e.g. animals, humans, seedlings) ▪ No relevant exposure (e.g. airborne contamination) ▪ Methodological articles (e.g. procedures for determination of fluoride in soil or plant material) ▪ Full text not available ▪ Not in English Third stage (assessing full text): ▪ Same criteria of the first and second stages ▪ No relevant outcome ▪ Other reasons

Available qualitative and quantitative information was extracted on the basis of the forms reported in Table 2 and Table 3 respectively. If the data were not available in the text, they were extracted from tables and graphs.

Table 2 - Qualitative information extracted from the studies

Variable	Description
Country	Country where the study was conducted
Zone	Specific study area (village or town, city, district, province etc.)
Type of experiment	Pot/Field/Hydroponic (P/F/H)
Growing conditions	Greenhouse/Open field/Growth Chamber (G/O/GC)
Substrate characteristics	Characteristics of soil/substrate or nutrient solution in which the plants were grown
Source of Exposure	Soil/Water (S/W)
Pollutant	Natural pollution, NaF, KF, NH_4F etc.
Other factors and levels	Other factors considered in the cases of factorial experiments and respective levels
Fertilization or amendment management	Type and doses of fertilizers/amendments applied
Species of plant	Scientific name of the studied plants
Variety	Varieties of the studied plants, if available
Phenology stage	Phenology stage at which plant where harvested
Available variables	F content in plant tissues (F), yields (Y), stress symptoms (S), others (O): specify
Accumulation pathway	Fluoride accumulation pathway among different plant organs
Effects of fluoride on plants	Visible injuries, oxidative stress, enzymatic activity etc.

Table 3 - Quantitative information extracted from the studies

Variable	Description
Sample size	Number of replicates per each treatment
Levels of fluoride exposure (ppm)	Different levels of concentration of fluoride in water or soil (ppm)
Levels of fluoride in different plant tissues (ppm)	For each observation means and standard deviation will be extracted
Yield of different plant parts (g/plant)	Dry matter of different plant parts or the entire plant biomass

Results and discussion

Overall results

A total of 85 studies were retrieved through the process of the systematic review, with the 75 % of the collected articles published after the year 2010.

Field-scale studies, pot experiments, hydroponic experiments and multi-experiments studies were found. Some of them reported exclusively data on fluoride accumulation in crops, from others it was possible to get only data on the effects of fluoride on plants and further articles included both type of data (Table 4).

Around 42 % of the studies were focused on soil pollution, 38 % on water pollution and the remaining 20 % on both sources of fluoride contamination.

Among the studies conducted under controlled conditions (pot and hydroponic experiments), in the 74% of the cases NaF was used as a pollutant to contaminate the source of exposure (soil or water), KF was used in the 11% of the studies, NH_4F in another 11% while AlF and HF in the 4%.

Table 4 - Number of studies collected per each category of experiment type and kind of extractable data.

Type of experiments conducted in the included studies	N. of studies that reported data exclusively on F accumulation	N. of studies that reported data or results exclusively on the effects of F on plants	N. of studies that reported both kind of data
Field-scale	32	2	3
Pot	11	10	18
Hydroponic	0	1	5
Field + Pot	1	0	0
Field + Hydroponic	1	0	0
Pot + Hydroponic	0	0	1

Fluoride in the water-soil-plant system of different areas of the world and human health hazard

The geographical distribution of the regions in which the fluoride in the water-soil-plant system has been investigated at field-scale so far, well reflects that of the high-fluoride groundwaters already reported by several authors (Ali, et al., 2016, Amini, et al., 2008, Edmunds and Smedley, 2013, Kimambo, et al., 2019) (Figure 1).

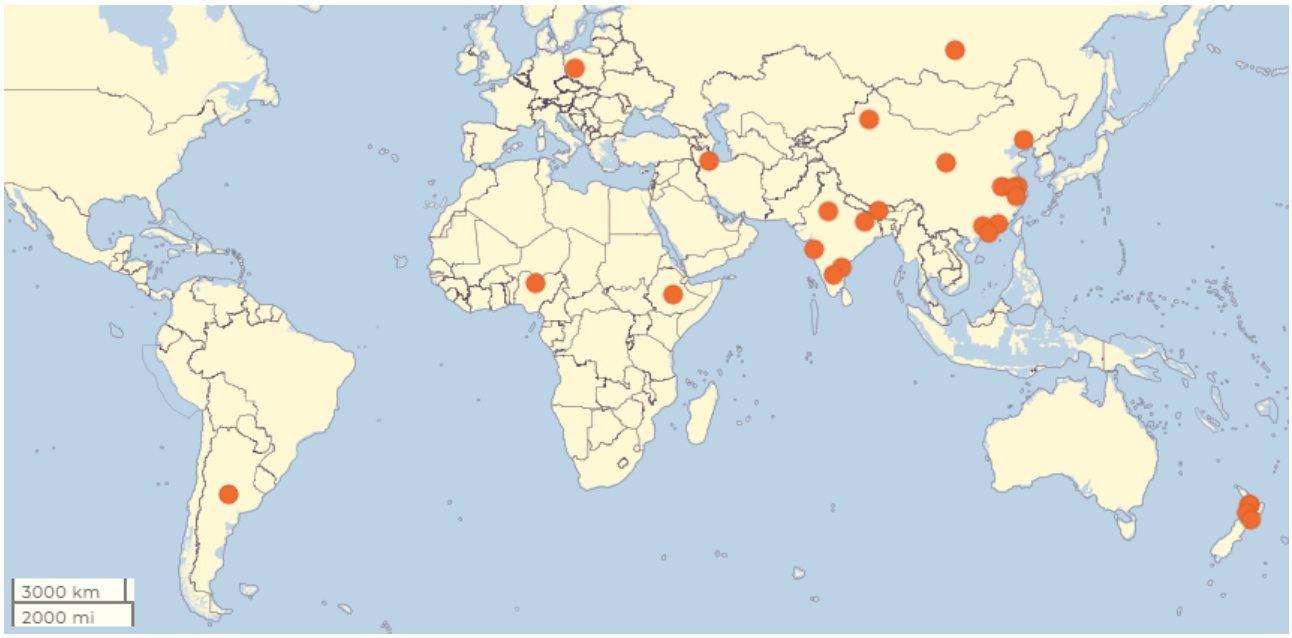

Figure 1 - Fluoride in agro-ecosystems: geographical distribution of the investigated areas (map was created using Mapline online free trial version: https://mapline.com).

The areas in which the greater numbers of studies have been conducted were India (16 studies) and China (11 studies), while just two field-scale studies were found for Pakistan and one field-scale study for each of the following regions was found: Argentina, Ethiopia, Iran, Nigeria, New Zealand, Poland and Russia (Table 5).

At a global level about 200 million people are estimated to be at risk of hazardous exposure to fluoride, 66 of which in India and 45 in China. A high prevalence was assessed also in South America and Africa (Edmunds and Smedley, 2013).

The analysis of the state of the art, thus, highlights that for some regions, such as for example the African Rift Valley, where groundwaters and surface waters recorded some of the highest

concentrations of fluoride ever observed (Davies, 2008, Edmunds and Smedley, 2013), the contribution of the local food and feed contaminated crops to the daily fluoride human exposure is still not fully investigated.

Concerning India, the majority of the studies regarded the areas of Rajasthan and West Bengal, furthermore other experiments were conducted also in Andhra Pradesh, Telangana, Bangalore, Uttar Pradesh and Bihar. Rightly these areas are recognised to be among the most affected of the country, first among all Rajasthan, where the risk of fluorosis concerns around 11 million people in 18 districts, and after that, Andhra Pradesh and Telangana which is the second-worst affected state in India (Ali, et al., 2016).

In general, the different studies considered a wide variety of food crops, including grain crops, pulses, horticultural crops, leafy vegetables and tubers.

The lowest value of soil total fluoride concentration (6.7 mg F^- kg^{-1}) was found by Nagaraju et al. (2017) in Talupula, Anantapur District, Andhra Pradesh while the highest (681.0 mg F^- kg^{-1}) was detected in Rajasthan by Saini et al. (2013). The soil water-soluble fluoride varied from 0.3 mg F^- kg^{-1} (Lakshmi, et al., 2016) in Aatmakoor Mandal, Nalgonda district, Telangana, to 73.9 mg F^- kg^{-1} in Rajasthan (Saini, et al., 2013). Concerning groundwater, the fluoride concentration ranged from 0.3 mg F^- L^{-1} in Birbhum district to 21.5 mg F^- L^{-1} in Bangalore (Begum, et al., 2008, Mondal and Gupta, 2015). The lowest contents of fluoride in various vegetable species (< 4.2 mg F^- kg^{-1} DM) were observed in the regions of Nowapara and Junidpur, Birbhum district and in Nalgonda district, Telangana (Lakshmi, et al., 2016, 2017a, 2017b, Pal, et al., 2012), while the greatest value was recorded in *Brassica oleracea* L. (296 mg F^- kg^{-1} DM) by Begum et al. (2008) in Bangalore.

Regarding China, endemic fluorosis has been documented almost in all provinces and the main sources of exposure are considered to be high fluoride water, pollution from coal-burning and an elevated consumption of brick tea (Kimambo, et al., 2019). Six out of the ten studies sited in China, in fact, concerned the uptake and accumulation of fluoride in tea plant (*Camellia sinensis* (L.) O.

Kuntze) that is well known to be one among the fluoride hyper-accumulator species (Cronin, et al., 2000). The concentration of fluoride in young tea leaves varied from a minimum of 49.0 mg F^- kg^{-1} DM found by Zhonglei et al. (2007), up to 808.0 mg F^- kg^{-1} DM observed by Fung et al. (1999) in Guangdong province. Mature leaves were found to have higher concentrations compared to young ones, ranging from 221.0 mg F^- kg^{-1} DM (Zhonglei, et al., 2007) to 2919.0 mg F^- kg^{-1} DM in Lantau Peak of Lantau Island (Fung, et al., 2003). The fluoride accumulation in edible parts of various food crops observed by different authors in China was always lower than 7.2 mg F^- kg^{-1} DM, however, the concentration of fluoride in wheat grains (0.6-7.2 mg F^- kg^{-1} DM) was found, in most of the cases, to be higher than the national edible health standard of China (CMH, 2012) ($\leq$ 1.5 mg kg^{-1}) (Li, et al., 2017, Li, et al., 2019, Wang, et al., 2012, Yu, et al., 2018, Zheng and Sun, 2011). Higher concentrations were found in roots, leaves and straw of wheat grown in the region of Baiyin, Gansu province and Yangtze River outfall region and in roots and leaves of maize, with the highest value (542 mg F^- kg^{-1} DM) observed in Gansu province in roots (Li, et al., 2017, Li, et al., 2019, Wang, et al., 2012). In this area also the highest concentration of fluoride were recorded both in soil (total fluoride: 4987 mg F^- kg^{-1}; water-soluble fluoride: 59 mg F^- kg^{-1}) and water (82 mg F^- L^{-1}) (Li, et al., 2017). The lowest values of soil total fluoride (82 mg F^- kg^{-1}) and water fluoride (0.2 mg F^- L^{-1}) were observed in Shihezi, Xinjiang by Yu et al. (2018) while the minimum soil water-soluble fluoride (0.04 mg F^- kg^{-1}) was found by Ruan and Wong (2001) in Jintan County (Jiangsu Province).

Environmental fluoride pollution of natural origin was also found in the water-soil-plant systems of Argentina, Ethiopia, Nigeria and Pakistan (Brahman, et al., 2014, Dagnaw, et al., 2017, De Troiani, et al., 1987, Kazi, et al., 2019, Okibe, et al., 2010), while anthropogenic sources of fluoride contamination were identified in Poland, New Zealand and Russia. In Poland the old Warta reservoir near the town of Luboń was severely contaminated by the wastes from a nearby chemical plant (Jezierska-Madziar and Pinskwar, 2003), in New Zealand Loganathan et al. (2001) studied the effects of the long-term application of P-fertilizer on the top-soil fluoride concentration (Table 5) while in

Russia Sokolova et al. (2019) studied the effects of fluoride contaminated soils 0.5 km from the IrkAZ (one of the largest enterprises in aluminium industry in Russia) on the productivity and fluoride uptake of some field crops.

Some of the abovementioned authors, in addition to assess the fluoride accumulation in food crops also conducted nutritional surveys among the populations of the contaminated areas in order to estimate a hypothetical fluoride daily ingestion (estimated daily intake, EDI) associated to the consumption of the studied crops and the related hazard index (HI) which represents how many times the effective intake exceeds the recommended dose. In many cases, the cumulative EDI from the diet and drinking water exceeded the recommended value (HI > 1) with children and teenagers being the categories considered at major risk (Bhattacharya, et al., 2017, Brahman, et al., 2014, Jha, et al., 2008a, Kazi, et al., 2019).

Table 5 - Fluoride contents in soil (TF = Total Fluoride, WSF = Water-Soluble Fluoride), water and vegetation in different areas of the world.

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
Argentina	Santa Rosa, La Pampa province	44.6-80.4	0.1-6.3	9.1	*Medicago sativa* L. (16.8-46.5)	De Troiani et al. (1987)
China	Anhui province	210.0-1138.0	0.5-5.8	-	*Camellia sinensis* (L.) O. Kuntze (56.0-350.0)	Cai et al. (2016)
	Central and southwest China	142.0-536.0	0.8-3.1	-	*Camellia sinensis* (L.) O. Kuntze (old leaves 221.0-1504.0; young leaves 49.0-602.0 stem 13.5-77.6)	Zhonglei et al. (2007)
	Guangdong province	186.2-387.9	0.8-2.7	-	*Camellia sinensis* (L.) O. Kuntze (mature leaves 463.0-1228.0; young leaves 340.0-808.0 fallen leaves 752.0-2315.0; branches 20.8-34.6 roots 23.5-37.3)	Fung et al. (1999)
	Hong Kong, Lantau Peak of Lantau Island (Ngong Ping of South Lantau)	319.5-370.7	1.4-3.0	-	*Camellia sinensis* (L.) O. Kuntze (old leaves 1317.0-2919.0; young leaves 250.0-356.0 branches 225.0-320.0 roots 214.0-380.0)	Fung et al. (2003)
	Shaoxing County, Zhejiang Province and Jintan County, Jiangsu Province	-	0.04-3.4	-	*Camellia sinensis* (L.) O. Kuntze (mature leaves 871.0-1337.0; shoot 95.0-175.0 twig 8.0-20.0 litter 111.0-574.0 roots 6.0-27.0)	Ruan and Wong (2001)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
	Mingshang county, Sichuan province	402.0	0.7-0.9 (*before remediation with polyphenol-Ce*); 0.4-0.5 (*after remediation with polyphenol-Ce*)	-	*Camellia sinensis* (L.) O. Kuntze (322.6-1028.7 *before remediation with polyphenol-Ce*; 167.9-259.8 *after remediation with polyphenol-Ce)*	Zhao et al. (2015)
	Liaohe River Basin	182.0- 1254.0	2.0-5.0	0.7-4.5	*Zea maize* L. (0.9-1.0), *Brassica rapa* L. *subsp. Pekinensis* (Lour.) Hanelt (0.5-3.0), *Raphanus sativus* L. (0.3-0.5)	Zheng and Sun (2011)
	Oasis region, Baiyin, Gansu province (Shapogang, Yaqushui, Sujiadun, Haojiachuan, Xidagou)	276.6-4986.7	2.7-59.3	0.33-81.7	*Triticum aestivum* L. (grains 1.2-2.9 leaves 11.2-91.9; stalks 2.1-11.4 husks 8.7-40.2 roots 14.8-539.4)	Li, et al. (2017)
	Baiyin, Gansu province (Dongdagou and Xidagou)	256-2664	17.0 - 216.6	-	*Triticum aestivum* L. (grains 6.0-7.2 leaves 86.6-185.9; stalks 13.6-19.7 husks 33.6-71.1 roots 49.3-207.8) *Zea maize* L. (grains 0.2-0.3 leaves 98-148; stalks 18-84 husks 5.0-7.0 roots 243-542)	Li, et al.(2019)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
	Shihezi, Xinjiang	81.6-991.2	1.6-32.8	Surface water 0.2-0.3 Grownwater 0.2-1.8	*Brassica pekinensis* (Lour.) Rupr. (1.9-2.2) *Cleome gynandra* L. (2.4), *Chrysanthemum coronarium* L. (1.8), *Phragmites australis* (Cav.) Trin. ex Steud (2.4), *Brassica chinensis* L. var. oleifera Makino et Nemoto (1.9), *Raphanus sativus* L. (2.3), *Amygdalus persica* L. var. compressa (Loud.) Yü et Lu (2.8), *Karelinia caspia* (Pall.) Less. (2.2)	Yu et al. (2018)
	Yangtze River outfall region	290.0-790.0	2.3-30.1	-	*Triticum aestivum* L. (grain 0.6-3.2 straw 1.2-13.1 roots 1.2-19.2)	Wang et al. (2012)
Ethiopia	Rift Valley and no Rift Valley areas	125.0-861.0	4.0-23.5	0.4-7.8	*Lactuca sativa* L. (2.9-5.8), *Beta vulgaris* L. var. cycla (L.) Ulrich (2.7-5.4) , *Brassica oleracea* L. var. capitata L. (2.1-2.7), *Brassica carinata* A.Braun (2.1-2.6)	Dagnaw et al. (2017)
India	Bangalore	-	-	4.8-21.5	*Brassica oleracea* L. (98-296), *Brassica rapa* L. (7.5-136.0), (2.5-145.5), *Cucurbita maxima* Duchesne (1.6-3.5)	Begum, et al. (2008)
	Gaya District, Bihar	F-endemic area 148.3-162.7 Control 41.7-109.7	F-endemic area 2.5-2.9 Control 0.4-0.5	-	*Triticum aestivum* L. (F-endemic 2.1-11.6, control 1.5-3.2), *Oryza sativa* L. (F-endemic 9.7–11.8, control 1.8-3.2), *Cajanus cajan* L. (F-endemic 1.1-2.1, control 1.4-3.5), *Cicer arietinum* L. (F-endemic 2.9-4.6, control .7-2.5), *Brassica nigra* (L.) W.D.J. Koch (F-endemic 3.8-4.4, control 2.8-4.0), *Solanum tuberosum* L. (F-endemic 0.5-0.7, control 1.4-2.6), *Spinacia oleracea* L. (F-endemic 3.6-4.8, control 1.6-4.2), *Solanum lycopersicum L.* (F-endemic 0.2-0.4, control n.a.), *Coriandrum sativum* L. (F-endemic 9.7-10.9, control 1.5-3.2)	Ranjan and Yasmin (2015)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
	Nalgonda district, Telangana, Aatmakoor mandal	-	0.3-2.6	0.7-4.7	*Oryza sativa* L. (0.3-2.2), *Sorghum bicolor* L. Moench. (0.3-2.1), *Cajanus cajan* L. (0.3-1.5), *Abelmoschus esculentus* L. (0.3-0.6), *Solanum lycopersicum L.* (0.2-0.9), *Brassica oleracea* L. (0.8), *Arachis hypogaea* L. (0.4-1.3)	Lakshmi et al. (2016)
	Nalgonda district, Telangana, Narkatpally mandal	-	0.3-2.6	0.6-5.3	*Oryza sativa* L. (0.3-2.3), *Sorghum bicolor* L. Moench. (0.2-1.8), *Cajanus cajan* L. (0.4-1.3), *Abelmoschus esculentus* L. (1.3-1.4), *Solanum lycopersicum L.* (1.0-1.3), *Arachis hypogaea* L.(0.5-2.4) L.,*Vigna radiata* (L.) R.Wilczek (0.2-1.4)	Lakshmi et al. (2017a)
	Nalgonda district, Telangana, Ramannapet mandal	-	0.4-2.4	0.5-3.9	*Oryza sativa* L. (0.1-2.2), *Sorghum bicolor* L. Moench. (0.3-1.6), *Cajanus cajan* L. (0.8-1.3), *Abelmoschus esculentus* L. (1.0-1.3), *Solanum lycopersicum L.* (0.4-0.8), *Brassica oleracea* L. (1.3), *Arachis hypogaea* L. (0.5-1.5), *Vigna radiata* (L.) R.Wilczek (1.5), *Capsicum frutescens* L. (1.4), *Solanum melongena* L. (0.6-1.3)	Lakshmi et al. (2017b)
	Northern periphery of the Lucknow	279.0-495.0	0.4-3.9	-	*Mentha arvensis* L. (66.2-110.5), *Spinacea oleracea* L. (28.6-69.1), *Luffa cylindrica* (L.) M.Roem. (12.0-26.6)	Jha et al. (2008a)
	Rajasthan	77.5-681.0	10.0-73.9	1.0-9.4	*Azadirachta indica* A.Juss. (46.7-56.3), *Prosopis juliflora* (Sw.) DC. (38.1-50.8), *Acacia tortilis* (Forssk.) Hayne (33.7-44.6), *Chenopodium album* L. (52.7-101.2), *Mentha arvensis* L. (33.7-55.6), *Spinacea oleracea* L. (29.3-66.2), *Brassica juncea* L. Czern. (8.8-15.4), *Triticum aestivum* L. (3.8-8.1), *Pennisetum glaucum* (L.) R.Br. (30.4-42.1)	Saini et al. (2013)
	Rajasthan Dausa, District	-	-	Growndwater 5.1-14.7	*Triticum aestivum* L.(3.2-14.3), *Solanum lycopersicum L.* (1.1-4.6), *Solanum tuberosum* L. (1.2-2.9)	Yadav et al. (2012)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
	Rajasthan, Nagaur district	-	-	1.6-14.6	*Pennisetum glaucum* (L.) R.Br. (1.7-7.4), *Hordeum vulgare* L. (1.8-5.9), *Cicer arietinum* L. (7.7-16.0), *Spinacea oleracea* L. (15.8-25.8), *Raphanus sativus* L. (10.3-22.4), *Brassica rapa subsp. campestris* (L.) A.R. Clapham (9.9-25.1), *Trigonella foenum-graecum* L.(18.1-19.1), *Brassica juncea* L. Czern. (14.3-14.6), *Triticum aestivum* L.(2.6-7.2), *Allium cepa* L. (7.9-24.1), *Chenopodium album* L. (13.0-14.1), *Cucumis melo* var. momordica (Roxb.) Duthie & J.B.Fuller (13.8-14.1), *Vigna radiata* L. R.Wilczek , (10.5-10.9), *Cyamopsis tetragonoloba* (L) Taub (13.1-13.4), *Pisum sativum* L.(8.2-8.5).	Gautam, et al. (2010b)
	Rajasthan, Sanganer tehsil in Jaipur district	-	-	2.3-13.0	*Triticum aestivum* L. (grains 3.6-22.3 leaves 1.2-4.7 roots 0.9-1.6)	Joshi and Bhardwaj (2012)
	Talupula, Anantapur District, Andhra Pradesh	6.7-8.8	-	-	*Helianthus annuus* L. (11.7-25.2), *Arachis hypogaea* L. (20.3-66.4) *Morus alba* L. (23.9-30.7) *Solanum lycopersicum L.* (11.0-18.3), *Phaseolus vulgaris* L. (17.8-26.8)	Nagaraju et al. (2017)
	West Bengal, Birbhum district, Junitpur village of Rampurhat block	146.4-178.0	6.3-17.4	0.3-4.8	*Triticum sp*. (14.9-28.9), *Oryza sativa* L. (14.6-17.5), *Brassica juncea* L. Czern. (20.2-44.2), *Allium cepa* L. (21.4-28.1), *Allium sativum* L. (8.1-29.3), *Daucus carota* L. (17.4-24.1), *Beta vulgaris* L. (17.3-21.2), *Pisum sativum* L. (24.9-28.8), *Cucumis sativus* L. (12.4-19.0), *Spinacea oleoracea* L. (44.8-57.9), *Apium graveolens* var. rapaceum (Miller) DC. (29.1-44.2), *Lens culinaris* Medik. (21.1-25.5), *Brassica oleracea* L. (20.0-30.6)	Mondal and Gupta (2015)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
	West Bengal, Bankura and Purulia districts	51.0-399.0	-	-	*Oryza sativa* L. (0.4-1.0), *Brassica juncea* L. Czern. (3.1-5.0), *Solanum tuberosum* L.(3.7-21.8), *Solanum lycopersicum* L.(5.7-11.2), *Brassica oleracea* L. var. Botrytis (10.5-22.9), *Brassica oleracea* L. var. Capitata (9.3-23.2), *Daucus carota* L. (40.9-74.0), *Raphanus sativus* L. (47.5-70.8), *Beta vulgaris* L. (5.5-12.7), *Spinacea oleracea* L. (3.3-6.7), *Vicia faba* L. (0.3-1.0), *Amaranthus sp*. (2.1-5.9), *Lagenaria siceraria* (Molina) Standl.(6.0-18.8), *Trigonella foenum-graecum* L. (0.7-2.7), *Coriandrum sativum* L. (11.0-21.0), *Cajanus cajan* L. (7.2-19.2), *Coccinia grandis* (L.) Voigt (0.3-0.5), *Solanum melongena* L. (16.4-21.6), *Brassica rapa subsp. Rapa* L. (0.5-1.5), *Allium cepa* L. (17.0-29.0)	Bhattacharya et al. (2017)
	West Bengal, Birbhum district, Junitpur village of Rampurhat block	-	1.5-3.8	0.6-4.1	*Brassica juncea* L. Czern. (4.2-4.6), *Solanum tuberosum* L.(3.8-4.2), *Spinacea oleracea* L. (11.1-11.7),*Coriandrum sativum* L. (26.8-27.1), *Marsilea sp.* (23.3-25.5), *Solanum lycopersicum* L. (8.6-8.9), *Solanum melongena* L. (13.4-15.5), *Allium cepa* L. (9.0-9.4), *Phaseolus vulgaris* L. (14.9-15.6)	Gupta and Banerjee (2011)
	West Bengal, Birbhum district, Junitpur village of Rampurhat block	139.2-144.8	1.5-3.8	0.7-4.1	*Oryza sativa* L. *Raphanus sativus* L. (grains 11.9-12.8 leaves 48.6-52.0 stem 18.8-20.4 roots 79.0-82.7)	Gupta and Banerjee (2009)
	West Bengal, Birbhum district, regions of Nowapara and Junidpur	-	-	0.5-10.2	*Brassica oleracea* L. (1.3), *Allium cepa* L. (3.2), *Raphanus sativus* L. (4.2), *Solanum tuberosum* L. (1.5), *Brassica oleracea* L. var. botrytis (1.1), *Spinacia oleracea* L. (2.6), *Carica papaya* L. (0.6), *Coccinia grandis* (L.) Voigt (0.8), *Solanum melongena* L. (1.4), *Dolichos lablab* L. (0.7), *Momordica charantia* L. (0.4)	Pal et al. (2012)

Country	Location	TF in soil (mg kg^{-1})	WSF in soil (mg kg^{-1})	F in water (mg L^{-1})	Cultivated species (mean or range F mg kg^{-1} DM)	References
Iran	Zanjan	-	0.4-1.2	-	*Solanum lycopersicum* L. (1.2-4.9), *Allium cepa* L. (1.1-3.7)	Moeinian et al. (2016)
New Zealand	Taranaki, Ruakura, Mid Canterbury, Hamilton, Woodville, Palmerston North	106.0-454.0	-	-	pastur mixed herbage (3-10)	Loganathan et al. (2001)
Nigeria	Bank of River Galma, Zaria	-	0.1-0.2	-	*Brassica oleracea* L. (0.06), *Daucus carota* L. (0.04), *Lactuca sativa* L. (0.1)	Okibe, et al. (2010)
Pakistan	Nagarparkar	125-566	3.9-18.6	18.5-35.4	*Phaseolus vulgaris* L. (25.0-38.7), *Pennisetum glaucum* (L.) R.Br. (19.4-33.4), *Vigna radiata* (L.) R.Wilczek (138.4-190.7)	Brahman, et al. (2014)
	Tharparkar	115-478	3.9-17.2	18.8-32.4	*Praecitrullus fistulosus (Stocks) Pangalo* (85.3–113.2), *Cucumis pubescens Willd.*(158–186), *Cyamopsis Tetragonoloba (L.) taub.)* (61.4–68.6)	Kazi, et al. (2019)
Poland	Old warta reservoirs near luboń and radzewice	-	-	-	*Phragmites australis* (Cav.) Trin. ex Steud. (Polluted reservoir: leaves 51.1, stalks 36.8,broots 213.0 Unpolluted reservoir: leaves 15.4, stalks 7.4, roots 5.4)	Jezierska-Madziar and Pinskwar (2003)
Russia	Irkutsk region	485-820	5-64	-	*Galega orientalis* Lam.(aboveground mass 47-50), *Melilotus officinalis* Lam. .(aboveground mass 44-61, roots 240-440), *Helianthus tuberosus* L. (aboveground mass 39-43, roots 45-47), *Zea mays* L. (aboveground mass 59-61, roots 59-110), *Avena sativa* L. (aboveground mass 41-51, roots 56-78), *Medicago sativa* L. (aboveground mass 39-45, roots 56-140), *Raphanus sativus var. oleifera* Metzg. (aboveground mass 37-76, roots 124-296)	Sokolova, et al (2019)

Fluoride uptake and partitioning under controlled conditions

Among the analysed publications concerning pot and hydroponic experiments, it was possible to get data on fluoride accumulation in plant tissues of 29 different vegetable species including cereals (*Hordeum vulgare* L., *Oryza sativa* L., *x Triticosecale* Wittm., *Triticum aestivum* L., *Zea mays* L.), pseudo-cereals (*Amaranthus gangeticus* L., *Amaranthus viridis* L.), pulses (*Glycine max* L. Merrill, *Lupinus angustifolius* L., *Lupinus luteus* L. *Onobrychis viciifolia* Scop.), horticultural species (*Abelmoschus esculentus* (L.) Moench), *Allium cepa* L., *Coriandrum sativum* L., *Ipomoea aquatica* Forssk., *Lactuca sativa* L., *Spinacea oleracea* L.), in particular species belonging to the Brassicaceae (*Brassica chinensis* L., *Brassica juncea* L., *Brassica napus* L., *Brassica oleracea* L., Raphanus sativus L.) and Solanaceae families (*Lycopersicon esculentum* L., *Solanum tuberosum* L.) and further cultivated species (*Camellia japonica* L., *Camellia sinensis* (L.) O. Kuntze, *Olea europaea* L., *Phacelia spp*. Juss., *Saccharum officinarum* L.) (Table 6).

Accumulation pattern

In general, for almost all the crops, roots were the organs that accumulated fluoride most. An exception was represented by tea plant and Japanese camellia that, as expected, hyper-accumulated fluoride in the leaves which showed higher concentrations compared to roots and young shoots (Camarena-Rangel, et al., 2015, JianYun, et al., 2004, Yang, et al., 2016); this trend was moreover confirmed also in the previously mentioned field-scale studies (Fung, et al., 1999, Fung, et al., 2003, Ruan and Wong, 2001, Zhonglei, et al., 2007). Another exception was represented by a study of Chen et al. (2017) on radish where the authors found that the accumulation of fluoride was highest in leaves than in roots. However, other studies conducted in radish confirmed the common higher concentration of fluoride in roots compared to leaves or, more generally, to aerial biomass (Chakrabarti and Patra,

2013a, Szostek and Ciecko, 2014). Furthermore, when mimicked sprinkler irrigation was applied (70-80% of water poured into the pot and 20-30% sprayed on plants) wheat (var. Raj. 3077) and barley (RD-2052) showed the highest concentration of fluoride in leaves, followed by stems, and then roots and grains (Agarwal and Chauhan, 2014, 2015). In other studies on cereals, roots were confirmed as the organs with the highest accumulation of fluoride, usually followed by the leaves and shoots and finally the grains (Chakrabarti, et al., 2013b, Jha, et al., 2013a, Mackowiak, et al., 2003, Szostek and Ciecko, 2014) or, in some other cases, as for example the studies of Bhargava and Bhardwa (2011) on wheat (var. Raj. 4083) and Gautam and Bhardwaj (2010a) on barley (var. RD-2683), with the grains or crop ears accumulating more fluoride than shoot and leaves, respectively.

In crops as lady's finger and sugar cane, leaves were the second organ for fluoride concentration followed by fruits and shoots (Lady's finger) or stem (sugar cane). At the opposite, in pakchoi, water spinach and lettuce, stem accumulated more fluoride than foliage (Zhang, et al., 2018). In mustard Ahmad et al. (2015) and Yadav et al. (2018) observed the following order: roots > seeds > shoots > leaves, while Chakrabarti and Patra (2013a) found a higher concentration of fluoride in leaves than in seeds. In olive tree the pattern was: roots > shoot > leaves > fruits (Zouari, et al., 2014). In onion, after roots, shoot accumulated more fluoride than bulb and in slender amaranth leaves accumulated more than seeds (Jha, et al., 2009, Stanley, et al., 2002).

In many species (winter oilseed rape, narrow-leaf lupine, yellow lupine, phacelia and spinach) the accumulation pattern roots > aerial mass was observed but the partitioning among aerial organs was not investigated (Jha, et al., 2008b, Szostek and Ciecko, 2014, YePu, et al., 2018). In some other case the accumulation pathway among organs was variable depending on the level of fluoride to which the plants were exposed (Bustingorri, et al., 2015, Bustingorri and Lavado, 2014, Das, et al., 2015).

Fluoride uptake

In the examined studies, an overall increasing trend of fluoride accumulation in organs of plants treated with rising concentration of fluoride in soil or water was observed.

The highest fluoride concentration (3217 mg F^- kg^{-1} DM) was found in rice roots in case of a hydroponic experiment; this species showed an hyper-accumulation also in the shoot (395 mg F^- kg^{-1} DM) with respect to the level of fluoride to which the plants were subjected (30 mg F L^{-1}) (Mackowiak, et al., 2003). Also tea leaves showed an elevated capacity to accumulate fluoride reaching up to 2553 mg F^- kg^{-1} DM in plants treated with 300 mg F^- kg^{-1} in soil (Yang, et al., 2016). Japanese camellia and sugar cane were also found to have high levels of fluoride in their tissues when treated with 30 mg F^- kg^{-1} of soil, up to 553 and 697 mg F^- kg^{-1} DM (Japanese camellia) and 521 and 120 mg F^- kg^{-1} DM (sugar cane) in roots and leaves, respectively. Considering its fast growth rate, sugar cane was best recommended by authors for phytoremediation purposes (Camarena-Rangel, et al., 2015). Very high concentrations were observed also by Zouari et al., (2014) in roots (up to 1070 mg kg^{-1} DM) and shoots (up to 570 mg F^- kg^{-1} DM) of olive tree treated with concentrations of fluoride in water up to 1900 mg F^- L^{-1}, and another extremely high value (1858 mg kg^{-1} DM) was observed by Elrashidi et al. (1998) in the biomass of barley cultivated in acid soil and treated with 400 mg F^- kg^{-1}.

Concerning cereals, when plants were treated with low concentrations of fluoride the lowest value (0.9 mg F^- kg^{-1} DM) was observed in grains of wheat irrigated with 4 mg F^- L^{-1} while, among plants treated with the highest concentrations, the maximum value (54 mg F^- kg^{-1} DM) was found in grains of rice plants treated with water at 30 mg F^- L^{-1} (Bhargava and Bhardwaj, 2011, Chakrabarti, et al., 2013b).

Among the Brassicaceae the lowest value (0.2 mg F^- kg^{-1} DM) was observed in cabbage treated with 10 mg F^- L^{-1} while the greatest (64 mg F^- kg^{-1} DM) was found in radish roots cultivated in soil with

300 mg F^- kg^{-1}. The maximum value for mustard seeds (22 mg F^- kg^{-1} DM) was found in the variety CS-14 irrigated with 75 mg F^- L^{-1}. Even they are not edible parts, it is worth noting that the shoots of winter oilseed rape and the leaves of radish reached 193 and 300 mg F^- kg^{-1} DM, respectively, when plants were grown in a soil contaminated with 1000 mg F^- kg^{-1} (Khandare and Rao, 2006, Szostek and Ciecko, 2014, Yadav, et al., 2018, YePu, et al., 2018).

Regarding pulses, values in soybean ranged between 0.4 and 9.0 mg F^- kg^{-1} DM, and 2.4 and 28 mg F^- kg^{-1} DM in seeds and leaves, respectively. Concentration of fluoride in the aerial mass of narrow-leaf lupine, yellow lupine and sainfoin varied from 3 to 8, 4 to 33 and 3 to 28 mg F^- kg^{-1} DM when plants were cultivated in soils contaminated with fluoride contents from 0 to 60, 0 to 300 and 0 to 150 mg F^- kg^{-1}, respectively (Bustingorri, et al., 2015, Bustingorri and Lavado, 2014, Szostek and Ciecko, 2014).

Among the leaf-vegetables (elephant-head amaranth, slender amaranth, coriander, water spinach, lettuce and spinach) very high values were reached in spinach (up to 220 mg F^- kg^{-1} DM) treated with 800 mg F^- kg^{-1} in soil (Jha, et al., 2008b). In lettuce, water spinach, coriander and elephant-head amaranth, treated with waters from 0 to 10 mg F^- L^{-1}, the concentrations of fluoride ranged respectively between 2.0-2.8, 4.3-6.2, 4.0-16.4 and 4.0-50.0 mg F^- kg^{-1} DM (Chakrabarti and Patra, 2013a, Khandare and Rao, 2006). Quite high concentrations were observed also in other horticultural species such as for example in potato tubers (up to 21 mg F^- kg^{-1} DM), lady's finger fruits (up to 49 mg F^- kg^{-1} DM) and onion bulbs (up to 54 mg F^- kg^{-1} DM) when exposed to the highest levels of fluoride (220, 600 and 800 mg F^- kg^{-1} of soil respectively) (Das, et al., 2015, Jha, et al., 2009, Jha, et al., 2013b).

Table 6 - Fluoride accumulation pattern among plant organs of different vegetable species exposed to increasing doses of soil or water fluoride.

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Abelmoschus esculentus (L.) Moench)	Parvani Kranti	Pot	Soil	NaF	**Soil:** 0, 100, 200, 300, 400, 500, 600 *fruits*: 39, 40, 40, 43, 43, 45, 49 *leaves*: 51, 57, 64, 72, 73, 76, 78 *shoots*: 26, 32, 33, 37, 40, 40, 41 *roots*: 17, 41, 45, 69, 76, 84, 106	root > leaves > fruits > shoot	Jha, et al. (2013b)
	-	Pot	Water	NaF	**Water:** 0, 10, 30, 60, 120 *Sand* *fruits*: 23, 4, 7, 22, 12 *leaves*: 46, 27, 96, 68, 165 *shoots*: n.a., n.a., 17, 9, n.a. *roots*: 71, 15, 90, 98, 59 *Soil* *fruits*: 24, 2, n.a., 15, n.a. *leaves*: 37, 23, 13, 26, 8 *shoots*: 15, 14, 13, 10, n.a. *roots*: 116, 63, 111, 65, 72	Sand: root/leaves/shoot > fruits Soil: root > leaves > fruit > shoot	Singh et al. (1995)
	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 0.1, 0.4	-	Khandare and Rao, (2006)
Allium cepa L.	Pusa Red	Pot	Soil	NaF	**Soil:** 0, 50, 100, 200, 400, 600, 800 *bulbs*: 16, 19, 19, 36, 49, 54 *shoots*: 16, 37, 43, 65, 96, 109 *roots*: 19, 46, 65, 102, 126, 152	root > shoot > bulb	Jha, et al., (2009)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Amaranthus gangeticus L.	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 4, 20	-	Khandare and Rao, (2006)
Brassica chinensis L.	-	Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *leaves*: 0.7, 1.4, 2.1, 3.0, 1.6, 1.3 *stem*: 1.6, 2.0, 3.3, 4.3, 3.5, 3.0 *roots*: 9, 10, 13, 16, 17, 15	root>stem>foliage	Zhang, et al., (2018)
Brassica juncea L.	-	Pot	Water	NaF	**Water:** 0, 3, 6, 9, 12, 15, 18, 21, 24 *seeds:* 0, 2.8, 2.5, 5.0, 7, 9, 10, 15, 17 *leaves*: 0, 2.6, 3.1, 4.4, 6.7, 7, 9, 12, 14 *shoots*: 0, 3.0, 3.8, 4.7, 5.7, 8, 9, 13, 16 *roots*: 0, 3.8, 5.2, 6.5, 8, 11, 12, 17, 19	roots > seeds > shoots/leaves	Ahmad, et al. (2015)
	Bio-902	Pot	Water	NaF	**Water:** 0, 25, 50, 75 *seeds*: 1.4, 7, 13, 16 *leaves*: 1.1, 6, 10, 11 *shoots*: 1.4, 6, 12, 14 *roots*: 1.6, 10, 15, 18	roots > seeds > shoots > leaves	Yadav, et al., (2018)
	Pusa-Tarak	Pot	Water	NaF	**Water:** 0, 25, 50, 75 *seeds*: 1.4, 10, 14, 18 *leaves*: 1.2, 8, 12, 14 *shoots*: 1.4, 8, 12, 18 *roots*: 1.6, 12, 17, 21	roots > seeds > shoots > leaves	Yadav, et al., (2018)
	CS-14	Pot	Water	NaF	**Water:** 0, 25, 50, 75 *seeds*: 1.5, 14, 19, 22 *leaves*: 1.2, 10, 14, 19 *shoot*: 1.4, 11, 16, 20 roots: 1.7, 15, 20, 25	roots > seeds > shoots > leaves	Yadav, et al., (2018)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
	Laxmi	Pot	Water	NaF	**Water:** 0, 25, 50, 75 *seeds*: 1.4, 6, 11, n.a. *leaves*: 1.1, 5, 6, n.a. *shoot*: 1.3, 6, 8, n.a. *roots*: 1.6, 9, 12, n.a.	roots > seeds > shoots > leaves	Yadav, et al., (2018)
	-	Pot	Water	NaF	**Water:** 0, 5, 10 *seeds*: 0, 2.7, 5.1 *leaves*: 0, 4.2, 8.2	leaves > seeds	Chakrabarti and Patra (2013a)
Brassica napus L.	-	Pot	Soil	NaF + soil natural F content (TF 313 ppm)	**Soil:** 0, 50, 100, 300, 500, 750, 1000 *shoots*: 42, 49, 55, 64, 73, 71, 193 *roots*: 154, 241, 277, 295, 324, 339, 357	roots > shoots	YePu, et al. (2018)
	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 1.5, 6, 13, 24 *roots*: 1.7, 37, 83, 119	roots > aerial mass	Szostek and Ciecko (2014)
Brassica oleracea L.	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 0.1, 0.2	-	Khandare and Rao (2006)
Camellia japonica L.	-	Hydroponic	Water	NaF	**Water:** 0, 2.5, 5, 10 *leaves*: 741, 768, 760, 697 *roots*: 273, 363, 275, 553	leaves > root	Camarena-Rangel, et al.(2015)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Camellia sinensis (L.) O. Kuntze	-	Pot	Soil	NH_4F	**Soil:** 0, 30 *leaves*: 1114, 1376 *young shoots*: 54, 96	leaves > young shoots	JianYun, et al. (2004)
	-	Pot	Soil	NH_4F	**Soil:** 0, 500 leaves (GR): 142, 212 leaves (IP): 139, 228 leaves (AR): 130, 198 leaves (VV): 135, 217 **soil taken from 4 different zones (GR, IP, AR, VV)	-	Sharma, et al. (2014)
	-	**Exp 1**: Hydroponic **Exp 3**: Pot	**Exp 1:** Water **Exp 3:** Soil	NaF	**Exp 1 water:** 0, 4.8, 9.5 *leaves*: 1508, 1815, 1784 *roots*: 30, 75, 35 **Exp 3 soil:** 0, 150, 300 *leaves*: 181, 651, 2553	leaves > roots	Yang, et al. (2016)
	-	Pot	Soil	NH_4F	**Soil** 0, 10, 20, 50, 100 *leaves*: 590, 760, 900, 1310, 1660 **Soil:** 0, 30 *leaves*: 570, 912 *young shoots*: 41, 67	leaves > young shoots	JianYun, et al. (2003)
	-	Hydroponic	Water	NH_4F	**Water:** 0, 3.8 *leaves*: 560, 771	-	Luo, et al. (2019)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Coriandrum sativum L.	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 4.0, 16.4	-	Khandare and Rao (2006)
	-	Pot	Water	NaF	**Water:** 0, 5, 10 *leaves*: 0, 22, 50	-	Chakrabarti and Patra (2013a)
Glycine max L. Merrill	Nidera 4613	Pot	Soil	NaF	**Soil:** 20, 25, 37, 200, 375, 450 *seeds*: 1.0, 1.2, 2, 3, 6 *pods*: 1.4, 2.5, 13, 16, 19 *leaves*: 2.4, 4.3, 13, 22, 28 *shoots*: 1.3, 2.1, 7, 16, 18 *roots*: 1.9, 3.4, 10, 25, 41	-	Bustingorri, et al. (2015)
	-	Pot	Water	NaF (mimicked sprinkler irrigation)	**Water:** 0, 4.5, 9, 25, 50, 200 *seeds*: 0.4, 2.6, 4, 6, 7, 8, 9 *pods*: 1.4, - , 2.3, 12, 14, 17 *leaves*: 2.4, - , 4.0, 12, 19, 23 *shoots*: 1.2, - , 1.9, 6, 15, 16 *roots*: 1.9, - , 3.1, 9, 23, 37	-	Bustingorri and Lavado (2014)
Hordeum vulgare L.	RD-2052	Pot	Water (mimicked sprinkler irrigation)	NaF 70% poured into the pot 30% sprayed on plant	**Soil:** 0, 3, 6, 9, 12, 15, 18 *seeds*: 0.4, 2.6, 4, 6, 7, 8, 9 *leaves*: 0.6, 3.5, 7, 10, 13, 14, 16 *shoots*: 0.5, 3.2, 6, 9, 11, 12, 14 *roots*: 0.4, 2.8, 5, 8, 10, 11, 13	leaves > shoot > root > grain	Agarwal and Chauhan (2015)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
	RD-2683	Pot	Water	NaF	**Water:** 0, 4, 8, 12, 15, 18, 20 *crop ear*: 0, 4, 5, 8, 10, 9, 14 *leaves*: 0, 3, 4, 7, 7, 8, 12 *shoots*: 0, 3, 4, 6, 8, 9, 13 *roots*: 0.2, 5, 7, 9, 10, 11, 17	roots > crop ear > shoots/leaves	Gautam and Bhardwaj (2010a)
	-	Pot	Soil	HF	**Soil:** 0,100,400, (1000 only alkaline soil) acid 50 P: 14, 64, 1858 acid 150 P: 15, 57, 964 acid 550 P: 14, 38, 771 neutral 50 P: 8, 43, 774 neutral 150 P: 6, 40, 459 neutral 550 P: 5, 24, 328 alkaline 50 P: 16, 29, 35, 64 alkaline 150 P: 12, 26, 41, 83 alkaline 550 P: 8, 21, 45, 105 *values referred to the total biomass ** P: Phosphorus content in soil (ppm)	-	Elrashidi, et al. (1998)
Ipomoea aquatica Forssk.		Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *leaves*: 4.3, 6.3, 7.3, 8.5, 7.6, 6.2 *stem*: 3.2, 4.4, 5.5, 5.2, 4.7, 3.7 *roots*: 11, 12, 16, 19, 20, 18	root > stem > foliage	Zhang, et al. (2018)
Lactuca sativa L.	-	Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *leaves*: 2.0, 2.2, 2.4, 2.8, 2.6, 2.0 *stem*: 3.0, 3.3, 3.7, 3.8, 4.4, 3.7 *roots*: 6, 8, 9, 10, 9, 9	root > stem > foliage	Zhang, et al. (2018)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Lupinus angustifolius L.	-	Pot	Soil	KF	**Soil:** 0, 20, 40, 60 *aerial mass*: 2.6, 3.4, 4.3, 8 *roots*: 1.8, 6.7, 12, 17	roots > aerial mass	Szostek and Ciecko (2014)
Lupinus luteus L.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 4.0, 13, 22, 33 *roots*: 1.9, 32, 49, 105	roots > aerial mass	Szostek and Ciecko (2014)
Lycopersicon esculentum L.	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 0.1, 0.2	-	Khandare and Rao (2006)
Olea europaea L.	Chemlali	Pot	Water	NaF	**Water:** 0, 380, 760, 1140, 1520, 1900 *fruits*: 11, 11, 11, 15, 29, 30 *leaves*: 12, 30, 71, 131, 221, 291 *shoots*: 9, 70, 141, 270, 471, 570 *roots*: 12, 290, 500, 739, 1000, 1070	roots> shoot> leaves> fruits	Zouari, et al. (2014)
Oryza sativa L.	-	Hydroponic	Water	KF	**Water:** 0; 9,5; 19; 38 *shoot*: 25, 72, 149, 395 *roots*: 48, 19, 541, 3217	root > shoot	Mackowiak, et al. (2003)
	IR-36	Pot	Water	NaF	**Water:** 0.3, 5, 10, 15, 20, 25, 30 *grains*: 0, 6, 10, 15, 21, 33, 54 *leaves*: 0, 6, 9, 14, 19, 30, 51 *roots*: 0, 6, 10, 15, 21, 33, 54	root > leaves > grains	Chakrabarti, et al. (2013b)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
	Swarno	Pot	Water	NaF	**Water:** 0.3, 5, 10, 15, 20, 25, 30 *grains*: 0, 2, 6, 10, 15, 25, 39 *leaves*: 0, 6, 9, 13, 19, 29, 49 *roots*: 0, 6, 10, 15, 20, 31, 51	root > leaves > grains	Chakrabarti, et al. (2013b)
	CSR23	Pot	Water	NaF	**Water:** 0, 2, 4, 8 *grains*: 12, 16, 18, 23 *straw*: 24, 30, 36, 44 *roots*: 18, 24, 34, 47	roots > straw > grains	Jha, et al. (2013a)
Onobrychis viciifolia Scop.	-	Pot	Soil	KF	**Soil:** 0, 50, 100, 150 *aerial mass*: 2.5, 7, 15, 28	-	Szostek and Ciecko (2014)
Phacelia spp. Juss.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 0.9, 6, 12, 26 *roots*: 1.3, 13, 44, 72	roots > aerial mass	Szostek and Ciecko (2014)
Raphanus sativus L.	-	Pot	Soil	NaF + soil natural F content (TF 265 ppm)	**Soil:** 0, 50, 100, 300, 500, 750, 1000 *leaves*: 67, 42, 103, 130, 146, 197, 300 *roots*: 6.7, 7.4, 18, 13, 15, 27, 19	leaves > roots	Chen, et al. (2017)
	-	Pot	Water	NaF	**Water:** 0, 5, 10 *leaves*: 0, 21, 47 *roots*: 0, 26, 60	roots > leaves	Chakrabarti and Patra (2013a)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 1.9, 19, 22, 28 *roots*: 2.2, 31, 61, 64	roots > aerial mass	Szostek and Ciecko (2014)
Saccharum officinarum L.	-	Hydroponic	Water	NaF	**Water:** 0, 2.5, 5, 10 *leaves*: 67, 55, 86, 120 *stem*: 52, 46, 48, 47 *roots*: 443, 985, 634, 521	root > leaves > stem	Camarena-Rangel, et al. (2015)
Solanum tuberosum L.	-	Pot	Soil	NaF	**Soil:** 11, 22, 44, 110, 220 *tubers*: 23, 6, 14, 14, 21 *leaves*: 24, 56, 15, 84, 19 *shoot*: 18, 21, 13, 29, 70 *roots*: 29, 22, 31, 21, 37	-	Das, et al. (2015)
Spinacea oleracea L.	Palak All Green	Pot	Soil	NaF	**Soil:** 0, 50, 100, 200, 400, 600, 800 *shoots*: 24, 50, 88, 104, 125, 167, 220 *roots*: 34, 58, 85, 102, 163, 214, 294	root > shoot	Jha, et al. (2008b)
	-	Pot	Water	NaF	**Water:** 0.3, 10 *edible part*: 0.7, 1.7	-	Khandare and Rao (2006)
	-	Pot	Water	NaF	**Water:** 0, 5, 10 *leaves*: 0, 9, 18	-	Chakrabarti and Patra (2013a)
x Triticosecale Wittm.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *straw*: 5, 8, 13, 31 *roots*: 3, 43, 59, 103	roots > straw	Szostek and Ciecko (2014)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure and concentration in plant tissues (ppm)	Accumulation pattern	Reference
Triticum aestivum L.	Raj. 4083	Pot	Water	NaF	**Water:** 0, 4, 8, 12, 16, 20 *grains*: 0, 0.9, 1.3, 1.7, 2.4, 2.9 *leaves*: 0, 0.5, 0.7, 0.9, 1.3, 1.5 *shoots*: 0, 0.9, 0.9, 1.5, 1.9, 2.1 *roots*: 0, 2.2, 2.6, 3.3, 3.8, 4.2	root > grain > shoot > leaves	Bhargava and Bhardwa (2011)
	Raj. 3077	Pot	Water (mimicked sprinkler irrigation)	NaF 80% poured into the pot 20% sprayed on plant	**Water:** 0, 3, 6, 9, 12, 15, 18, 21 *grains*: 1.0, 3.1, 4, 6, 8, 9, 10, 11 *leaves*: 2.8, 5.9, 9, 13, 15, 17, 19, 21 *stem*: 2.1, 4.6, 7, 10, 12, 14, 15, 17 *roots*: 1.3, 3.6, 6, 9, 11, 12, 14, 15	leaves > stem > root/grains	Agarwal and Chauhan (2014)
	KRL19	Pot	Water	NaF	**Water:** 0, 2, 4, 6, 8 *grains*: 12, 13, 14, 15 *straw*: 22, 33, 40, 53 *roots*: 25, 37, 48, 62	roots > straw > grains	Jha, et al. (2013a)
Zea mays L.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 1.8, 2.7, 11, 19 *roots*: 2.1, 24, 43, 125	roots > aerial mass	Szostek and Ciecko (2014)

Effects of fluoride on plants

Effects on growth and biomass

A decrease in the total plant biomass of plants exposed to rising concentrations of fluoride was observed in many crops such as barley, rice, wheat, lady's finger, onion, soybean, radish, spinach, triticale and olive tree (Table 7). In contrast, Szostek and Ciecko (2017b) found that increasing levels of soil fluoride contamination (up to 300 mg F^- kg^{-1} of soil) stimulated the biomass produced by radish, phacelia, narrow-leaf lupin, yellow lupin, winter oilseed rape and aerial parts of maize plants while the production of spring triticale straw and grains, maize roots, and aerial parts of lucerne was depressed (Table 7).

In species as wheat, soybean, black gram, onion, radish, olive tree, lady's finger and tea a reduction in shoot and root length was also highlighted (Ahmed, et al., 2019b, Bhargava and Bhardwaj, 2011, Bustingorri, et al., 2015, 2017, Jayakumar, et al., 1997, Yang, et al., 2016) accompanied in some cases from inhibition of germination, and reduction in leaf size, number of flowers, number of seeds and 1000-grain weight (Singh, et al., 2001, 2013, Zouari, et al., 2016).

In tea plants, at the opposite, Sharma et al. (2014) and Camarena-Rangel et al. (2015) did not observe significant effects on shoot height, root length, leaf area, number of leaves, fresh leaf weight and dry leaf weight compared to control.

Table 7 - Variation in dry biomass (%) of different vegetable species exposed to increasing doses of soil or water fluoride.

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure (ppm) and production (g plant^{-1} DM unless otherwise specified)	Percentage of yield increase (+) or decrease (-) with respect to control (%)	Reference
Abelmoschus esculentus (L.) Moench)	-	Pot	Water	NaF	**Water:** 0, 10, 30, 60, 120 *Sand* *biomass*: 1.3, 1.6, 1.3, 1.4, 0.5 *Soil* *biomass*: 1.7, 1.2, 2.4, 1.3, 1.0	**Water:** 10, 30, 60, 120 *Sand* *biomass*: +23, 0, +8, -62 *Soil* *biomass*: -29, +41, -24, -41	Singh, et al. (1995)
	Parvani Kranti	Pot	Soil	NaF	**Soil:** 0, 100, 200, 300, 400, 500, 600 *biomass*: 13.4, 12.8, 11.2, 11.0,10.2, 9.8,9.5	**Soil:** 100, 200, 300, 400, 500, 600 *biomass*: -4, -16, -18, -24, -27, -29	Jha, et al. (2013b)
	Nirali	Pot	Water	NaF	**Water:** 0, 50, 100, 150, 200, 250, 300 *seeds*: 7.8, 5.9, 4.0, 2.8, 2.1, 1.2, 1.0 *pods*: 2.5, 2.4, 2.2, 2.0, 1.4, 1.3, 1.1	**Water:** 50, 100, 150, 200, 250, 300 *seeds*: -24, -49, -64, -73, -85, -88 *pods*: -4, -12, -20, -44, -48, -56	Ahmed, et al. (2019a)
	Arka Anamika	Pot	Water	NaF	**Water:** 0, 50, 100, 150, 200, 250, 300 *seeds*: 10.7, 6.2, 3.0, 1.8, 1.3, 1.0, 0.9 *pods*: 2.5, 2.5, 2.3, 2.2, 1.6, 1.3, 1.2	**Water:** 50, 100, 150, 200, 250, 300 *seeds*: -42, -72, -83, -88, -91, -92 *pods*: 0, -8, -12, -36, -48, -52	Ahmed, et al. (2019a)
Allium cepa L.	Pusa Red	Pot	Soil	NaF	**Soil:** 0, 50, 100, 200, 400, 600, 800 *bulbs*: 3.3, 3.2, 3.2, 2.7, 1.4, 1.0 *shoots*: 2.5, 2.5, 2.5, 2.0, 1.2, 0.7 *roots*: 0.5, 0.5, 0.5, 0.3, 0.2, 0.1	**Soil:** 50, 100, 200, 400, 600, 800 *bulbs*: -3, -3, -18, -58, -70 *shoots*: 0, 0, -20, -60, -72 *roots*: 0, 0, -40, -60, -80	Jha, et al. (2009)
Brassica chinensis L.	-	Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *biomass:* 0.4, 0.6, 0.8, 1.1, 1,0, 0.8	**Water:** 0.4, 1, 3, 6, 10 *biomass*:+50,+100,+175,+150,+100	Zhang, et al. (2018)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure (ppm) and production (g plant^{-1} DM unless otherwise specified)	Percentage of yield increase (+) or decrease (-) with respect to control (%)	Reference
Brassica napus L.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 44.8, 60.4, 73.4, 72.5 *roots*: 3.3, 4.1, 4.8, 5.2 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 0, 100, 200, 300 *aerial mass*: +35, +64, +62 *roots*: +24, +45, +58	Szostek and Ciecko (2017b)
	-	Pot	Soil	NaF and soil natural pollution	**Soil:** 0, 50, 100, 300, 500, 750, 1000 *biomass:* 6.5, 5.5, 3.8, 3.6, 2.2, 1.8, 1.1	**Soil:** 50, 100, 300, 500, 750, 1000 *biomass*: -15,-42, -45, -66, -72, -83	Li and Chenb (2018)
Camellia sinensis (L.) O. Kuntze	-	**Exp 1**: Hydroponic **Exp 3**: Pot	**Exp 1:** Water	NaF	**Exp 1 water:** 0, 4.8, 9.5 *roots*: 0.4, 0.4, 0.5	**Exp 1 water:** 4.8, 9.5 *roots*: -0, +25	Yang, et al. (2016)
			Exp 3: Soil		**Exp 3 soil:** 0, 150, 300 *leaves*: 2.4, 2.3, 1.9	**Exp 3 soil:** 150, 300 *leaves*: -4, -21	
	-	Hydroponic	Water	NH_4F	**Water:** 0, 3.8 *leaves*: 0.9, 0.8	**Water:** 3.8 *leaves*: -11	Luo, et al. (2019)
Glycine max L. Merrill	Nidera 4613	Pot	Soil	NaF	**Soil:** 20, 37, 200, 375, 450 *seeds*: 4.8, 4.4, 3.5, 3.6, 3.2 *leaves*: 5.1, 5.0, 4.8, 3.8, 3.5 *shoots*: 5.5, 6.0, 5.4, 4.6, 4.1 *roots*: 8.4, 7.3, 6.3, 4.9, 3.6	**Soil:** 37, 200, 375, 450 *seeds*: -8, -27, -25, -33 *leaves*: - 2, -6, -25, -31 *shoots*: +9, -2, -16, -25 *roots*: -13, -25, -42, -57	Bustingorri, et al. (2015)
	Nidera 4613	Pot	Soil	NaF	**Water:** 0, 4.5, 9, 25, 50, 200 *seeds*: 4.7, 4.8, 4.4, 3.5, 3.4, 3.2 *pods:* 3.1, 3.3, 2.9, 1.9, 1.8, 1.5 *leaves*: 3.9, 2.5, 3.0, 2.0, 1.9, 1.5 *shoots*: 4.8, 4.7, 4.6, 2.4, 2.3, 1.7 *roots*: 4.2, 4.4, 4.0, 2.2, 1.7, 1.1	**Soil:** 37, 200, 375, 450 *seeds*: 2, -6, -26, -28, -32 *pods:* 6, -6, -39, -42, -52 *leaves*: -36, -23, -49, -51, -62 *shoots*: -2, -4, -50, -52, -65 *roots*: 5, -5, -48, -60, -74	Bustingorri and Lavado (2014)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure (ppm) and production (g plant^{-1} DM unless otherwise specified)	Percentage of yield increase (+) or decrease (-) with respect to control (%)	Reference
Hordeum vulgare L.	-	Pot	Soil	HF	**Soil:** 0,100,400, (1000 only alkaline soil) acid 50 P: 3.5, 2.0, 1.0 acid 150 P: 4.1, 3.0, 1.1 acid 550 P: 3.7, 3.4, 0.8 neutral 50 P: 3.3, 4.4, 3.3 neutral 150 P: 4.4, 3.7, 2.9 neutral 550 P: 4.7, 4.9, 3.2 alkaline 50 P: 6.7, 7.3, 7.0, 6.4 alkaline 150 P: 6.5, 6.0, 5.8, 6.6 alkaline 550 P: 6.9, 6.0, 6.4, 6.4 *values referred to the total biomass ** P: Phosphorus content in soil (ppm)	**Soil:** 100,400, (1000 alkaline soil) acid 50 P: -43, -71 acid 150 P: -27, -73 acid 550 P: -8, -78 neutral 50 P: +33, -0 neutral 150 P: -16, -34 neutral 550 P: +4, -32 alkaline 50 P: +9, +4, -4 alkaline 150 P: -8, -11, +2 alkaline 550 P: -13, -7, -7	Elrashidi, et al. (1998)
Ipomoea aquatica Forssk.		Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *biomass:* 0.4, 0.6, 0.5, 0.4, 0.3, 0.3	**Water:** 0.4, 1, 3, 6, 10 *biomass*: +50, +25, 0, -25, -25	Zhang, et al. (2018)
Lactuca sativa L.	-	Pot	Water	NaF	**Water:** 0, 0.4, 1, 3, 6, 10 *biomass:* 0.5, 0.9, 1.2, 1.4, 1.4, 1.0	**Water:** 0.4, 1, 3, 6, 10 *biomass*:+80,+140,+180,+180,+100	Zhang, et al. (2018)
Lupinus angustifolius L.	-	Pot	Soil	KF	**Soil:** 0, 20, 40, 60 *aerial mass*: 48.9, 78.3, 66.6, 69.5 *roots*: 12.4, 17.1, 16.5, 20.1 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 20, 40, 60 *aerial mass*: +60, +36, +42 *roots*: +38, +33, +62	Szostek and Ciecko (2017b)
Lupinus luteus L.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 28.5, 32.9, 32.7, 32.2 *roots*: 4.0, 4.1, 4.7, 4.2 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 100, 200, 300 *aerial mass*: +15, +15, +13 *roots*: +2, +18, +5	Szostek and Ciecko (2017b)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure (ppm) and production (g plant^{-1} DM unless otherwise specified)	Percentage of yield increase (+) or decrease (-) with respect to control (%)	Reference
Olea europaea L.	Chemlali	Pot	Water	NaF	**Water:** 0, 380, 760, 1140, 1520, 1900 *leaves*: 11.2, 11.5, 10.7, 9.0, 7.0, 6.2 *shoots*: 16.9, 16.9, 16.0, 15.0, 13.0, 12.0 *roots*: 10.9, 10.5, 10.3, 9.4, 8.9, 8.6	**Water:** 380, 760, 1140, 1520, 1900 *leaves*: +3, -4, -20, -38, -45 *shoots*: 0, -5, -11, -23, -29 *roots*: -4, -6, -14, -18, -21	Zouari, et al. (2014)
Oryza sativa L.	CSR23	Pot	Water	NaF	**Water:** 0, 2, 4, 8 *biomass*: 18.2, 18.1, 17.9, 17.7	**Water:** 2, 4, 8 *biomass*: -1, -2, -3	Jha, et al. (2013a)
Phacelia spp. Juss.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 207.7, 209.5, 207.8, 207.0 *roots*: 6.7, 7.6, 7.6, 8.3 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 0, 100, 200, 300 *aerial mass*: +0.9, 0.0, -0.3 *roots*: +13, +13, +24	Szostek and Ciecko (2017b)
Raphanus sativus L.	-	Pot	Soil	NaF + soil natural F content (TF 265 ppm)	**Soil:** 0, 50, 100, 300, 500, 750, 1000 *biomass*: 9.9, 7.1, 4.4, 4, 6.8, 4.2, 3.8	**Soil:** 50, 100, 300, 500, 750, 1000 *biomass*: -28, -56,-60,-31,-58, -62	Chen, et al. (2017)
	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 189.3, 193.5, 192.2, 204.7 *roots*: 83.2, 83.8, 86.8, 87.8 * g pot^{-1} FM (8 plant pot^{-1})	**Soil:** 100, 200, 300 *aerial mass*: +2, +2, +8 *roots*: +1, +4, +6	Szostek and Ciecko (2017b)
Spinacea oleracea L.	Palak All Green	Pot	Soil	NaF	**Soil:** 0, 50, 100, 200, 400, 600, 800 *shoots*: 4.1, 4.1, 4.0, 3.8, 3.7, 3.5, 3.4 *roots*: 1.1, 1.0, 1.0, 1.0, 0.9, 0.8, 0.7	**Soil:** 50, 100, 200, 400, 600, 800 *shoots*: -0, -2, -7, -10, -15, -17 *roots*: -9, -9, -9, -18, -27, -36	Jha, et al. (2008b)

Species	Variety	Pot/ Hydroponic	Source Soil/Water	Pollutant	Levels of fluoride exposure (ppm) and production (g plant^{-1} DM unless otherwise specified)	Percentage of yield increase (+) or decrease (-) with respect to control (%)	Reference
Triticum aestivum L.	HD 2329	Field	Water	NaF	**Water:** 0, 25, 50, 100, 200 *seeds*: 6.0, 5.8, 4.9, 3.2, 3.0	**Water:** 0, 25, 50, 100, 200 *seeds*: -3, -18, -47, -50	Singh, et al. (2001)
	Raj. 4083	Pot	Water	NaF	**Water:** 0, 4, 8, 12, 16, 20 *grains*: 0.9, 0.8, 0.8, 0.8, 0.8, 0.7 *shoots*: 0.6, 0.6, 0.5, 0.5, 0.5, 0.5 *roots*: 0.9, 0.9, 0.8, 0.8, 0.8, 0.8	**Water:** 4, 8, 12, 16, 20 *grains*: -11, -11, -11, -11, -22 *shoots*: 0, -17, -17, -17, -17 *roots*: 0, -11, -11, -11, -11	Bhargava and Bhardwaj (2011)
	KRL19	Pot	Water	NaF	**Water:** 0, 2, 4, 8 *biomass*: 11.2, 10.9, 10.1, 9.4	**Water:** 2, 4, 8 *biomass*: -3, -10, -16	Jha, et al. (2013a)
x Triticosecale Wittm.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *seeds*: 40.5, 35.8, 30.3, 26.6 *straw*: 45.0, 43.4, 42.5, 41.5 *roots*: 21.6, 19.6, 19.0, 18.6 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 0, 100, 200, 300 *seeds*: -12, -25, -34 *straw*: -4, -6, -8 *roots*: -9, -12, -14	Szostek and Ciecko (2017b)
Zea mays L.	-	Pot	Soil	KF	**Soil:** 0, 100, 200, 300 *aerial mass*: 570.7, 594.3, 585.8, 594.4 *roots*: 150.4, 131.1, 115.3, 135.4 * g pot^{-1} FM (13 plant pot^{-1})	**Soil:** 100, 200, 300 *aerial mass*: +4, +3, +4, *roots*: -13, -23, -10	Szostek and Ciecko (2017b)

Visible symptoms

In species as onion, tip burning was observed (Jha, et al., 2009) while in wheat plants, chlorosis and necrosis symptoms were detected (Joshi and Bhardwaj, 2012). Leaf necrosis was found also by Yang et al. (2016) in tea plants treated with 300 mg F^- kg^{-1} of soil and visual symptoms of fluoride toxicity appeared as well in olive leaves in which necrosis was apical and/or marginal and progressively extended over the whole surface of the leaf lamina (Zouari, et al., 2014). On the other hand, Bustingorri et al. (2015) in soybean, Jha et al. (2013a, 2008b) in rice and wheat, and JianYun et al. (2003) in spinach and tea did not find visible evidence of fluoride toxicity.

Oxidative stress and defensive mechanisms

Superoxide dismutases (SODs) are prevalent metalloenzymes greatly involved in the immune response of living organisms. They are regarded as the first important defence enzymes within a plant cell being able to eliminate the oxidants in the cell parts affected (Ismail and Suroto, 2012), particularly toxic superoxide radicals (ROS). Biochemical changes observed in plants under fluoride stress may be related to the antioxidative defence mechanisms operating in the plant cells (Chakrabarti and Patra, 2015). The formation and accumulation of toxic oxygen species, like hydrogen peroxide (H_2O_2), increasing in lipid peroxidation (LP) and electrolyte leakage (EL) has been associated with various type of stresses (HuiMei, et al., 2016). Other studies monitored the thiobarbituric acid reactive substances (TBARS) production as a marker of free radical formation in plant tissues. Moreover, under the influence of different environmental stresses, other studies reported a reduction in glutathione (GSH) (Snioszek, et al., 2018), ascorbic acid (AA), total flavonoid (Flav) and total polyphenol content (Phe). Antioxidant enzymes like catalase (CAT), glutathione peroxidase (GPOX) and ascorbate peroxidase (APX) can catalyse the rupture of hydrogen peroxide H_2O_2.

Among the analysed studies, various authors observed an increase in SOD activity when plants were exposed to fluoride (Bustingorri, et al., 2017, Chakrabarti and Patra, 2013a, Chakrabarti and Patra, 2015, Jha, et al., 2013a, Snioszek, et al., 2018) but numerous other studies showed that whereas lower fluoride concentrations stimulated the antioxidant system, higher fluoride levels caused a significant reduction of antioxidant enzyme activities, carotenoids and other elements with an increase of oxidative stress indicators such as H_2O_2, TBARS, LP and EL both in roots and leaves (Chakrabarti and Patra, 2015, HuiMei, et al., 2016, Zouari, et al., 2017). In a study of Bustingorri et al. (2017) on soybean a significant decrease in GSH and GPOX with respect to control was observed only at high fluoride concentration, with an increase of TBARS formation in leaves and no TBARS increase in roots. Furthermore, Snioszek et al., (2018) found significant alterations in the level of AA, GSH, Flav and Phe in pea plants cultivated in contaminated soils, compared to the control plants.

Effects on chlorophyll

Total chlorophyll reduction is primarily caused by the chlorophyll collapse or inhibition of chlorophyll synthesis due to fluoride stress (Chang and Thompson, 1963). In many of the analysed studies was found that the chlorophyll content of fluoride-treated plants decreased with respect to controls in a dose-dependent manner (Ahmed, et al., 2019b, Bustingorri, et al., 2017, Chakrabarti and Patra, 2015, Jha, et al., 2013a, Joshi and Bhardwaj, 2012, Sharma, et al., 2018, Zouari, et al., 2016). HuiMei et al. (2016) observed a decrease in photosynthetic and chlorophyll fluorescence parameters at fluoride doses higher than 5 mg L^{-1}. Joshi and Bhardwaj (2012) reported a modest effect of fluoride on Chlorophyll b and a significant reduction in Chlorophyll a in plants irrigated with increasing levels of fluoride. Furthermore, Camarena-Rangel, et al. (2015) found that the chlorophyll decrease was manifested after 28 days for all the fluoride levels tested suggesting that this parameter could be a better indicator of cellular integrity than others such as oxidation or wilting. In contrast, Stanley et al.

(2002) found that the chlorophyll a and b of leaf samples did not show any statistical difference in the tested species.

Nutrients balance

Zouari et al., (2017) suggested that the increased uptake of some minerals observed in olive could be interpreted as a defence response of plants to fluoride. Szostek and Ciecko (2017a) observed that the concentration of calcium and magnesium was highly affected by the soil contamination with fluorine, with reductions in the average content of calcium in the roots of yellow lupin and winter rape by 63 and 42%, respectively, and of magnesium in the roots of spring triticale by 42% with respect to the control plants. In contrast, tea plants subjected to fluoride treatments did not show any significant alteration in the concentrations of macro and microelements (K, Ca, Mg, S, P, Fe, Mn, Zn) in leaves (JianYun, et al., 2003, 2004).

Further effects

An interesting strategy to adapt and resist to high concentrations of fluoride was observed by HuiMei et al. (2016) in leaves of tea plants consisting in the reduction in stomatal aperture with an increase in the epidermal hairs number. Similar results were found in young olive trees under fluoride stress, in which slight reductions of leaf relative water content (LRWC) and stomatal conductance were detected (Zouari, et al., 2016). Moreover, among the effects caused by fluoride on plants, a serious damage to the ultrastructure of the cells was observed through transmission electron microscopy within a study in tea plant conducted by Li and Chenb (2018).

Conclusions

The outcomes of this systematic review emphasized that food and feed crops cultivated in areas affected by soil and water fluoride pollution may reach levels of fluoride concentration potentially harmful to human health. Several of the examined studies, in fact, highlighted that the fluoride intake deriving from those foods might significantly increase the daily fluoride ingestion of individuals, contributing, together with the consumption of contaminated water, to the risk of overcoming the recommended dose and enhancing the related hazard of incurring fluorosis diseases.

However, in spite of the efforts that were already made in the assessment of crop fluoride accumulation for some contaminated areas, mostly Asia, this review suggests that further studies are needed to analyse the fluoride in food crops particularly in those regions where the environmental fluoride contamination is a serious issue and the prevalence of fluorosis is well documented, such as for example the East African Rift Valley, from which just one field-scale study was retrieved through this review process.

From the analysis of literature on controlled condition experiments, it came out that, with few exceptions, plants tend to accumulate fluoride mostly in the root system and this can represent a disadvantage in the case of crops whose edible part is represented by the underground organs, as for example radish. Nevertheless, the accumulation of fluoride in other edible parts (e.g. leaves, fruits or seeds) can reach considerable levels when plants are exposed to increasing doses of fluoride. Many authors observed the manifestation of toxic effects of fluoride on plants which was found in fact to induce oxidative stress, reduction in chlorophyll content, alterations in the level of AA, GSH, Flav, Phe and macro and micronutrients. In spite of that, the manifestation of symptoms such as visible injuries, decreasing in root and shoot length and yield reduction was not always observed, also at high doses of fluoride exposure, and in some cases the biomass production was even stimulated by increasing fluoride levels.

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Annex 1 - Review Protocol

Objective

The main objective is to assess, based on the available scientific literature, the impacts of soil and water fluoride pollution on the safety and productivity of food and feed crops at a global scale.

Research questions:

Which is the fluoride uptake capacity and partitioning of different kind of crops cultivated in soils and/or irrigated with water characterized by various levels of fluoride contamination?

Which are the effects of high fluoride levels in the soil and/or in the irrigation water on quali-quantitative characteristics of crop production for different type of crops?

Table 1a. Population, Exposure, Comparator and Outcome

Population	Exposure	Comparator	Outcome
Cultivated plants	Fluoride concentration in the soil or in the irrigation water	different vegetable species	uptake capacity and partitioning change
Cultivated plants	Fluoride concentration in the soil or in the irrigation water	No/less fluoride in the soil or in the irrigation water	quali-quantitative characteristics of crop production change

The search aims to find all available publications, including data from field-scale explorative trials and experiments under controlled conditions, assessing the impacts of soil and water fluoride contamination on the fluoride uptake capacity and partitioning and quali-quantitative production features of food and feed crops.

Methodology

Electronic searches will be performed in scientific databases and will be oriented by established guidelines (Bilotta, et al., 2014, Collaboration for Environmental Evidence, 2013, Pullin and Stewart, 2006, Pullin and Stewart, 2009)[1].

Academic search engines will comprise: ISI Web of Science (Web of Science Core Collection plus other databases such as BIOABS, KJD, MEDLINE, RSCI, SCIELO), PubMed, Scopus, Science Direct, JSTOR, Agricola, Fluorideresearch.org, Cab Abstracts.

Search terms and strings

Advanced search tools will be used for conduct the systematic search entering the following keywords:

fluoride OR fluorine (in the title field)
AND
crop OR plant OR vegetation OR grasses OR vegetables OR agriculture (in the others fields)

- Languages: English
- Timespan: 1985-2019

To refine the scoping results the search conducted on the Web of Science Core Collection and the Scopus database will be restricted to the following subject areas:

- *for the Web of Science Core Collection*: Environmental sciences, Toxicology, Water resources, Plant sciences, Soil science, Agronomy, Ecology, Agriculture multidisciplinary, Multidisciplinary sciences, Biology, Forestry, Horticulture Agricultural engineering, Physiology, Agriculture dairy animal science, Environmental studies
- *for the Scopus database*: Environmental Science, Agricultural and Biological Sciences, Multidisciplinary, Decision Sciences

[1] Bilotta, G.S., A.M. Milner and I. Boyd. 2014. On the use of systematic reviews to inform environmental policies. Environmental Science Policy 42: 67-77.
Collaboration for Environmental Evidence. 2013. Guidelines for Systematic Review and Evidence Synthesis in Environmental Management. Version 4.2. Environmental Evidence: 1-81
Pullin, A.S. and G.B. Stewart. 2006. Guidelines for systematic review in conservation and environmental management. . Conservation Biology 20: 1647-1656.
Pullin, A.S. and G.B. Stewart. 2009. Doing more good than harm - building an evidencebase for conservation and environmental management. Biological Conservation 142: 931- 934.

The final numbers of articles recovered, duplicated, accepted and excluded will be annotated. Records will be stored in a reference manager software database and duplicates will be eliminated.

Inclusion and exclusion criteria

The article screening will be performed following a stepwise process according to the established inclusion criteria. A first screening will be conducted on the base of the publication title, after this, abstracts will be evaluated and finally, the remaining articles will be filtered revising the whole paper. In case of doubts about the pertinence of a record, it will be included and further evaluated in the next step. To assess the reliability of application of the inclusion criteria, a second and a third reviewer will revise a subset of the references (Pullin & Stewart 2006).

Inclusion criteria

Every kind of study assessing:

- the effects of soil and water fluoride on quali-quantitative characteristics of crop production
- fluorine uptake capacity of different kind of crops cultivated in soils and/or irrigated with water characterized by various levels of fluoride
- articles focusing only on adults plants
- microcosm, mesocosm and field-scale trials

Exclusion criteria

First and second stages (screening title and abstract):

- Not on topic (misclassified)
- No relevant population (e.g. animals, humans, seedlings)
- No relevant exposure (e.g. airborne contamination)
- Methodological articles (e.g. procedures for determination of fluoride in soil or plant material)
- Full text not available
- Not in English

Third stage (assessing full text):

- Same criteria of the first and second stages
- No relevant comparator
- No relevant outcome
- Other reasons

Data extraction

Available qualitative information will be extracted according to the following scheme:

Table 2a - Qualitative information that will be extract from the studies

Variable	Description
Country	Country where the study was conducted
Zone	Specific study area (village or town, city, district, province etc.)
Type of experiment	Pot/Field/Hydroponic (P/F/H)
Growing conditions	Greenhouse/Open field/Growth Chamber (G/O/GC)
Substrate characteristics	Characteristics of soil/substrate or nutrient solution in which the plants were grown
Source of Exposure	Soil/Water (S/W)
Pollutant	Natural pollution, NaF, KF, NH_4F etc.
Other factors and levels	Other factors considered in the cases of factorial experiments and respective levels
Fertilization or amendment management	Type and doses of fertilizers/amendments applied
Species of plant	Scientific name of the studied plants
Variety	Varieties of the studied plants, if available
Phenology stage	Phenology stage at which plant where harvested
Available variables	F content in plant tissues (F), yields (Y), stress symptoms (S), others (O): specify
Accumulation pathway	Fluoride accumulation pathway among different plant organs
Effects of fluoride on plants	Visible injuries, oxidative stress, enzymatic activity etc.

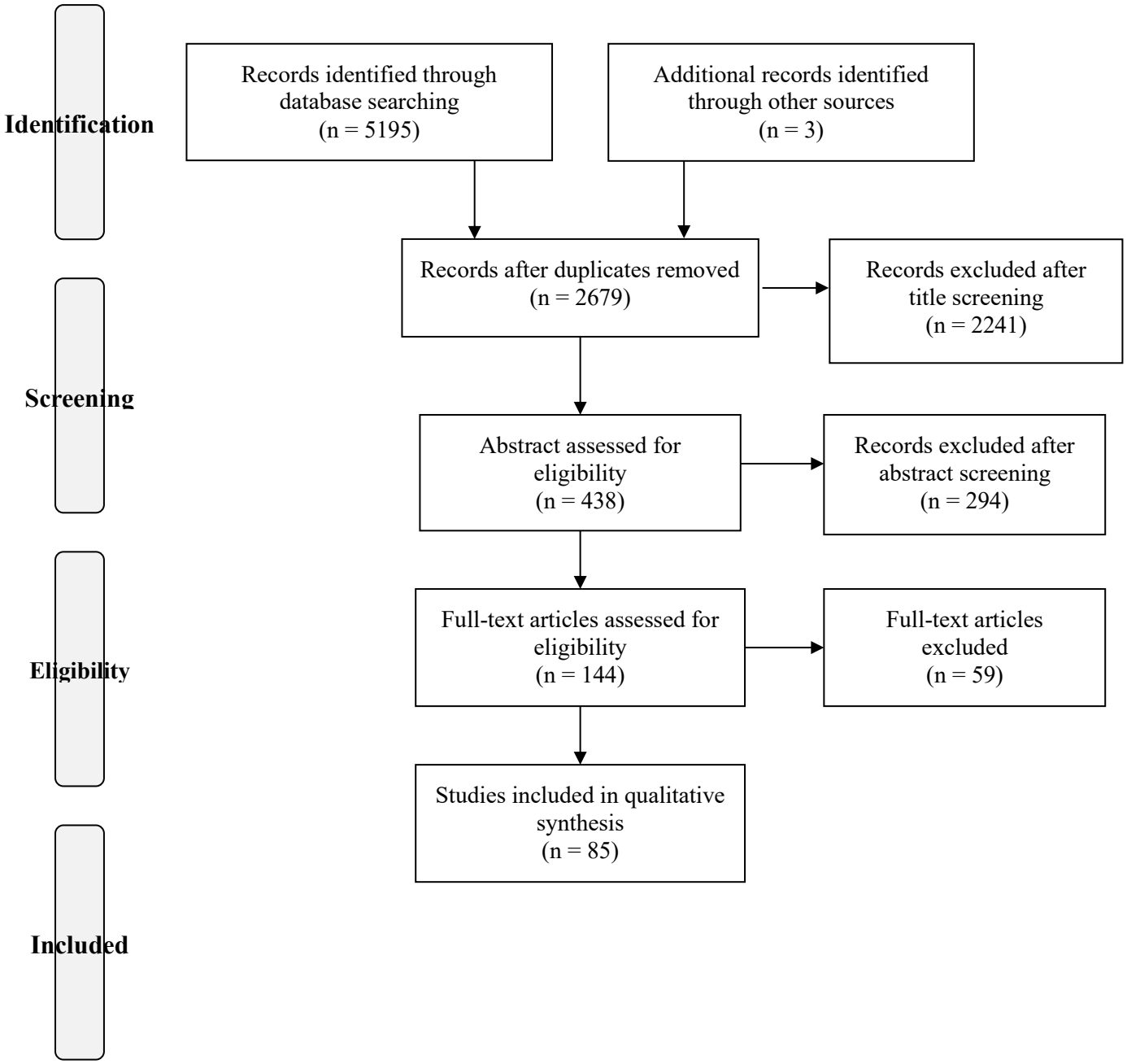
Available quantitative information will be extracted according to the following scheme:

Table 3a - Quantitative information that will be extract from the studies

Variable	Description
Sample size	Number of replicates per each treatment
Levels of fluoride exposure (ppm)	Different levels of concentration of fluoride in water or soil (ppm)
Levels of fluoride in different plant tissues (ppm)	For each observation means and standard deviation will be extracted
Yield of different plant parts (g/plant)	Dry matter of different plant parts or the entire plant biomass

If the data is not available in the text, it will be extracted from tables and graphs.

Annex 2 - Flow Diagram (Modified from The PRISMA Group 2009)[2].



[2] From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Annex 3 - List of publications included in the systematic review

Agarwal, R. and S.S. Chauhan. 2014. Bioaccumulation of sodium fluoride toxicity in *Triticum aestivum* var. Raj. 3077. Interantional Journal of Food, Agriculture and Veterinary Sciences 4: 98-101.

Agarwal, R. and S.S. Chauhan. 2015. Bioaccumulation of sodium fluoride toxicity in plant parts at different phases and its impact on crop *Hordeum vulgare* (Barley) Variety RD 2052. International Journal of Multidisciplinary Research and Development 2: 16-21.

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Bhargava, D. and N. Bhardwaj. 2011. Phytotoxicity of fluoride on a wheat variety (*Triticum aestivum* var. Raj. 4083) and its bioaccumulation at the reproductive phase. Journal of Experimental Sciences 25: 37-40.

Bhattacharya, P., A.C. Samal, S. Banerjee, J. Pyne and S.C. Santra. 2017. Assessment of potential health risk of fluoride consumption through rice, pulses, and vegetables in addition to consumption of fluoride-contaminated drinking water of West Bengal, India. Environmental Science and Pollution Research International 24: 20300-20314.

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Chakrabarti, S. and P.K. Patra. 2015. Biochemical and Antioxidant Responses of Paddy (*Oryza sativa* L.) to Fluoride Stress. Fluoride 48: 56-61.

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Chapter II

Fluoride uptake and translocation in food crops grown in fluoride-rich soils

Fluoride uptake and translocation in food crops grown in fluoride-rich soils

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Running title: Fluoride uptake of food crops grown in fluoride-rich soils

Abstract

The East African Rift Valley (EARV) area is characterized by an intense volcanic activity, which largely influences the nature of soils, ground and surface waters causing a transfer of fluoride from volcanic emissions to the environment. Field experiments were conducted in F-contaminated areas of Ngarenanyuki (Arumeru district) in North Tanzania. In order to evaluate the potential fluoride exposure from the diet and the related health risk for the local population, the content of fluoride in soil and plant tissues was assessed, focusing on the edible portions (leaves, fruits or seeds) of the main cultivated and consumed food crops in the area. Average fluoride contents of 14.2, 11.4, 11.3 and 8.0 ppm were observed respectively for kale, tomato, bean and maize edible parts.

The cumulative estimated average daily dose (EADD) ranged from 0.026 to 0.165 mg F d^{-1} kg^{-1} among different population groups and considering two different hypotheses of absorption fraction (75 % or 100 %), i.e. the amount of fluoride that is absorbed during the digestion process. The associated hazard index (HI) values varied from 0.43 to 2.75.

Considering the dietary habits of the local population, the outcomes of the present study suggest that the investigated crops can substantially contribute to fluoride related diseases, especially in earlier ages.

Keywords: Maize, Tomato, Bean, Kale, Soil contamination, East African Rift Valley, Translocation Factor, Hazard Index

Introduction

The East African Rift Valley (EARV) is among the few active rifts on the Earth's surface characterized by intense volcanic activity. Direct impacts of such volcanism include the rise, from the lower crust or upper mantle, of volatile and potentially harmful elements and their release to groundwater. These peculiar geological features have a great influence on the nature of the soils and the quality of water and food (Davies, 2008). Particularly concerning fluorine (F), Kloos and Haimanot (1999) reported that the concentrations of this element in the volcanic rocks of the East African Rift System are higher than those of the same kind of rocks in other regions. Some of the highest fluoride concentrations in groundwater ever recorded in the world were detected in the EARV (Edmunds and Smedley, 2013), such as 100 mg L^{-1} or more measured in Tanzania by Ghiglieri et al. (2012). In these areas, high concentrations of fluoride were also registered in surface water, up to 700 mg L^{-1} in the Momela Lake. The Engare Nanyuki river, that is locally utilized as a source of irrigation since it is the only perennial stream in the East and North-East of Mount Meru, showed about 30 mg L^{-1} of fluoride up to 60 mg F^{-} L^{-1} at its spring (Ghiglieri, et al., 2012). These concentrations can vary during the year due to different climatic conditions, as extreme evaporation has a consistent influence on the salt concentration.

For over a hundred years, researchers from different disciplines studied the relationship between environmental fluoride contamination and health status of the population living in these so-called endemic areas (Ozsvath, 2009). Excessive and continuous intake of this element in the human diet can lead, in fact, to a series of severe diseases known as fluorosis (Frencken, 1992). The fluoride introduced with the diet is assimilated into teeth and bones (Kebede, et al., 2016) forcing out hydroxide ions from hydroxyapatite crystal with the formation of a tougher and stronger new mineral: fluorapatite (Theiss, et al., 2014). Even if a small concentration (1.3 mg L^{-1}) of fluoride intake is considered beneficial for the reduction of dental decay and the promotion of proper bone

development, the ingestion of higher concentrations of fluoride can result into dental caries, mottled staining of teeth and malformations in bone structures (Davies, 2008). Fluorosis can affect both young and old people. Fluoride ingestion during pregnancy and breastfeeding could affect the nervous system of the developing foetuses and of the infants leading to future behavioural disorders and reduced IQ (Begum, et al., 2008, Lu, et al., 2000).

Endemic fluorosis is a worldwide problem. Some countries such as Mexico are moderately affected while in other countries such as India, Tanzania, Ethiopia, Kenya and Argentina, fluoride contamination in groundwater is a crucial matter (Ali, et al., 2016). In EARV about 90% of the population had shown fluorosis symptoms (Yoder, et al., 1998) and in the region of Arusha in Tanzania the prevalence of dental fluorosis is about 100% (Vuhahula, et al., 2009). The Tanzanian national standard for drinking water has been raised up to 4 mg L^{-1} respect to the WHO limit of 1.5 mg L^{-1}, reflecting the difficult situation of both pollution of groundwater and water scarcity (Edmunds and Smedley, 2013).

Most health studies regard chronic fluoride exposure from drinking water. However, food can also be responsible for another potentially significant pathway (Ozsvath, 2009). Fluoride can be uptaken by food and feed crops through soil solution and gaseous emissions, entering the man food chain because of the consumption of contaminated vegetables and maybe also of livestock products about which very few data are present in scientific literature. Nevertheless, human health problems associated to the food chain are still poorly investigated, thus, the determination of fluoride intake from the daily diet is extremely valuable for enhancing our understanding of the role of food in fluorosis occurrence (Gupta and Banerjee, 2011).

The main natural source of fluoride in the agricultural soil is represented by the disruption of fluoride-rich minerals from the bedrock (Pickering, 1985). Other important sources are the irrigation water derived from groundwater and surface water as well as the surface runoff from contaminated sites to

downstream areas (Bustingorri, et al., 2015). Fluoride can then be detained in a durable way in soils and it is primarily associated with the colloid or clay fractions. For all soils, it is the soluble fluoride content which is biologically active for plants and animals (Loganathan, et al., 2006, Loganathan, et al., 2006). Plants roots passively absorb the soluble fluoride through the soil water solution and then it is moved via xylematic conducts up to the stems, leaves and fruits (Ahmad, et al., 2018). The bioaccumulation of fluoride can vary in the different plant parts, depending on both the transfer from soil solution to roots and the translocation from roots to shoots (Bhargava and Bhardwaj, 2011), but many studies highlighted a higher concentration of fluoride in roots than the other plant parts (Baunthiyal and Ranghar, 2014, Chakrabarti, et al., 2013b, Lakshmi, et al., 2017a, Takmaz-Nisancioglu and Davison, 1988).

The aims of this study were (i) to assess the concentration of fluoride content in the edible portions (leaves, fruits or seeds) of the main cultivated and consumed crops (bean, kale, maize, tomato) of the study area in the Rift Valley of Northern Tanzania; (ii) to investigate the partitioning of fluoride among the different organs of the plant in order to calculate the bioconcentration and translocation factors.

Materials and methods

Study area

The study area is located in Northern Tanzania, in the Arusha Region, Arumeru District, within the Ngarenanyuki ward (about 22'130 ha) (Oikos, 2011). Three different sites within this main region were identified as representatives of the whole area mainly according to the willingness of the householder to participate in the experiment and the reliability of the information provided: Uwiro (3°9'28.00"S, 36°51'23.10"E), Olkung'wado (3°11'20.42"S, 36°51'17.30"E) and Momela (3°12'54.50"S, 36°51'39.30"E).

The soil characteristics of the study sites are reported in Table 8. Soil textures vary from loamy sand to sandy loam. Hydrological parameters were derived following Saxton (2006) on the basis of the soil texture, organic matter content and soil salinity. The soil pH varied from moderately alkaline to strongly alkaline. Regarding soil fertility, Uwiro was the site with the highest content of organic carbon, total nitrogen and assimilable phosphates. Momela and Olkung'wado soils showed a low C/N ratio and low Olsen P content. In addition, the organic C in Olkung'wado was poor. All experimental sites showed an average CEC and a high to a very high content of exchangeable Na and K. Momela and Uwiro were poor in exchangeable Ca while Olkung'wado was very poor. Exchangeable Mg was poor in Momela, average in Uwiro and very poor in Olkung'wado. Mg/K ratio was very low for all the three sites.

Regarding salinity, Momela soil is considered non-saline, Uwiro slightly saline and Olkung'wado from slightly to strongly saline. Considering the sodicity, soils were sodic in Uwiro and Olkung'wado while normal in Momela.

As regards the climatic features, the mean temperatures in the case study areas range from 11.6°C in July to 27.7°C in February. The annual mean temperature is 18.9°C and the hottest and coldest season fall between January and February and June to August respectively. With an Aridity Index (AI) of

0.63, the climate of the study area can be classified as dry-sub-humid (CLIMATE-DATA.ORG©, 2019).

Two main rainy periods characterize the rainfall pattern of the region. The first, between the ends of February to mid-May, is known as "Masika" or long rainy period and is characterized by regular rains (545 mm/year). The second one, in October-December, present a higher variability of rain intensity and distribution and it is named "Vuli" or short rainy season (210 mm/year). In the rest of the year, dry periods prevail (Agrawala, et al., 2003). The investigation described in this work was conducted during the long rainy season from March to August 2018.

The choice of the crops to be investigated was the outcome of 120 questionnaires collected locally on cropping and livestock systems and dietary behaviour at household scale (Rizzu, et al., 2017). The survey highlighted that most of the agricultural land in the three sites was cultivated with maize (*Zea mays* L.) as most common crop, followed by tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris* L.) and an ecotype of kale (*Brassica sp. pl.*) locally known as "Sukuma wiki",. According to Roser and Ritchie (2019), the average human daily energy requirement in Tanzania is 2208 kcal d^{-1} per-capita (updated to 2013). With respect to this value, the average daily consumptions of the four considered food items, as emerged from the survey, represent 26.3 %, 0.2 %, 6.8 % and 1.5 %, of the energy consumption respectively.

Table 8 - Soil characteristics of the three study sites at the beginning of the experiments

Study site	*Momela*		*Uwiro*		*Olkung'wado*		*Reference of the analytical method*
Depth (cm)	*0-20*	*20-40*	*0-20*	*20-40*	*0-20*	*20-40*	
Particle size classes (%)							Astm D422 and UNI CEN ISO/TS 17892-4
Coarse sand > 100 µm	71.8	70.1	52.9	52.2	51.7	43.4	
Fine sand 100-50 µm	13.5	10.0	20.6	16.7	20.1	20.3	
Coarse silt 50-20 µm	6.2	6.7	10.2	10.5	13.3	15.4	
Fine silt 20-2 µm	8.5	8.1	6.7	12.9	10.7	14.8	
Clay < 2 µm	0.1	5.1	9.8	7.9	4.2	6.2	
Wilting Point (% vol)	3.0		9.5		7.7		
Field Capacity (% vol)	9.3		18.2		13.0		
Available Water (cm cm^{-1})	3.2		4.4		2.6		
pH	8.3	8.7	8.7	9.1	10.0	10.1	ISO 10390
Organic C (g kg^{-1})	11.7	4.0	24.7	17.7	5.9	3.9	ISO 10694
Total N (g kg^{-1})	1.7	0.6	2.6	2.0	1.1	0.9	Method XIV.1 G.U. 248 21/10/1999
C/N	7.1	6.6	9.6	8.8	5.1	4.3	
Olsen phosphorus (P_2O_5, mg kg^{-1})	9.2	9.2	128.3	96.3	9.2	18.4	Method XIV.1 G.U. 248 21/10/1999
CEC (meq 100 g^{-1})	11.7	14.6	12.6	20.6	11.0	14.7	ISO 11260
Exchangeable Ca (meq 100 g^{-1})	5.1	13.0	9.4	8.8	3.4	4.2	ISO 11260
Exchangeable Mg (meq 100 g^{-1})	0.8	0.8	1.8	1.6	0.3	0.5	ISO 11260
Exchangeable Na (meq 100 g^{-1})	1.8	1.7	4.3	6.5	6.7	5.9	ISO 11260
Exchangeable K (meq 100 g^{-1})	2.0	1.9	6.4	9.3	7.4	6.7	ISO 11260
Mg/K	0.4	0.4	0.3	0.2	0.0	0.1	
EC sat ext dS m^{-1} 25 °C	0.8	0.7	3.1	3.1	2.8	9.7	ISO 11265
Soluble Ca in H_2O (1:5) (mg kg^{-1})	13.8	12.5	60.1	71.9	31.3	30.7	Method IV.3 G.U. 248 21/10/1999
Soluble Mg in H_2O (1:5) (mg kg^{-1})	2.0	1.5	5.5	4.8	3.3	1.8	Method IV.3 G.U. 248 21/10/1999
Soluble Na in H_2O (1:5) (mg kg^{-1})	92.5	75.0	330.0	380.0	310.0	1145.0	Method IV.3 G.U. 248 21/10/1999
Soluble K in H_2O (1:5) (mg kg^{-1})	50.0	40.0	227.6	226.9	181.9	565.7	Method IV.3 G.U. 248 21/10/1999
ESP (%)	15.0	12.0	35.5	32.5	61.5	40.0	Method IV.3 G.U. 248 21/10/1999

Experimental layout and crop management

Every crop was replicated three times in each of the three selected sites within the study area, giving a total of 36 experimental units (3 sites x 4 crops x 3 replicates).

Table 9 - Plot size for each crop.

Crop	Plot size (m x m)	Space inter row (m)	Space between plants within row (m)	Nr. plants per plot	Nr. plants m^{-2}
Maize cv. Situka	5 x 6	0.75	0.30	48	4.4
Bean local ecotype	5 x 2	0.25	0.25	64	16.0
Tomato cv. Rio Grande	5 x 4	0.50	0.25	64	8.0
Kale local ecotype	5 x 4	0.50	0.30	48	6.7

In each of the three sites, maize and bean were manually seeded at the end of March 2018, while for tomato and kale a nursery using as substrate the soil of the corresponding area was prepared in middle April 2018 and the seedlings were transplanted after one month. Agricultural practices such as fertilization, weeding and application of pesticides were implemented according to similar business as usual practices in all the study sites by the local farmers; hence, since the experiment was conducted during the long rainy season, irrigation was not applied. Localized fertilization was done after one month from the seeding/transplanting with urea manually applied, following the business as usual fertilization practices.

Plant sample collection

At the maturity stage, for each crop, four randomly chosen plants per plot (one plant per row) were harvested and partitioned into roots, stems, leaves and grains/fruits. Plant samples were oven-dried for 72 hours at 60ºC and powdered with a Retsch ZM 200 (Retsch, Haan, Germany).

Fluoride determination in soil and plants

Water-soluble fluoride content in soil

For fluoride extraction, 2 g of sieved soil were shaken in deionised water (40 ml in a 50 ml Conical Centrifuge Tube, ratio 1:20) for 16 - 18 h. After the agitation period, the extraction fluid/sample mixture was centrifuged and the water-soluble fluoride was determined potentiometrically using a fluoride ion-selective electrode (ISE - Thermo Scientific Orion 9609BNWP). Both standards for the instrument calibration and samples were mixed 1:9 with a total ionic strength adjustment buffer (TISAB III) prior to analysis in order to adjust ionic strength, buffer pH to 5.0-5.5, and break up metal-fluoride complexes. In the case of soil samples with a lower fluoride content (<0.4 ppm) the accuracy of the results obtained with the ISE was verified on a series of samples by the ionic chromatography method.

Total fluoride content in plant samples

Plant powder samples were digested by Milestone Ethos Easy (Milestone s.r.l, Sorisole (BG), Italy) with nitric acid 67% (2 ml for each sample), hydrogen peroxide at 30% (3 ml for each sample) and deionised water (5 ml per sample), followed by neutralization with aqueous NaOH (8M). Prior to the neutralization, samples were immersed in a refrigerated bath (-30 °C) for 30 min in order to reduce the vapour pressure of nitric acid and avoid consequent losses of fluorine in the form of HF.

The neutralization was carried out directly inside the vessels immediately after their opening in order to rapidly stop the leakage of nitrous fumes with consequent loss of analyte.

With a strong NOx development, H_2O_2 addition allows a better digestion quality and at the same time a considerable reduction of NOx formation:

$$2NO_x + 3H_2O_2 \rightleftharpoons 2HNO_3 + 2H_2O$$

The chemical balance of the digestion reaction is shifted to the right, which usually increases the speed and quality of the digestions.

The fluoride concentration of the samples was determined potentiometrically using a fluoride ion-selective electrode (ISE - Thermo Scientific Orion 9609BNWP).

Both standards for the instrument calibration and samples were mixed 1:9 with a total ionic strength adjustment buffer (TISAB) prior to analysis in order to adjust ionic strength, buffer pH to 5-5.5, and break up metal-fluoride complexes.

Data analyses

Bioconcentration Factor (BCF) and translocation factor (TF)

A common index for estimating the fluoride concentration in plants is the bioconcentration factor (BCF) which is the ratio of fluoride concentration in the plant (or its component) and fluoride concentration in soil. Another factor that focuses on the partitioning of fluoride in the various vegetable organs and mainly in the translocation of fluoride within the plant is the translocation factor (TF), explained as the ratio of fluoride concentration in the entire shoot system (ESS) or edible part (EP) and the fluoride concentration in roots (Bustingorri and Lavado, 2014):

$$BCF = \frac{F\ concentration\ in\ ESS\ or\ plant\ part\ (\frac{mg\ F^-}{kg\ of\ DM})}{Water\ soluble\ F\ concentration\ in\ soil\ (\frac{mg\ F^-}{kg\ of\ soil})}$$

$$TF = \frac{F\ concentration\ in\ ESS\ or\ EP\ (\frac{mg\ F^-}{kg\ of\ DM})}{F\ concentration\ in\ roots\ (\frac{mg\ F^-}{kg\ of\ DM})}$$

Exposure dose: human fluoride exposure related to the investigated food items

For each study area, the individual fluoride exposure dose, coming from the four food items, was estimated according to the following equation, readapted from (USEPA, 1992):

$$EADDfood = \frac{C \times IR \times AF}{BW} \text{ (mg d}^{-1}\text{ kg}^{-1}\text{)}$$

where C is the concentration of fluoride in maize/bean/tomato/kale in the different study areas (mg kg-1 DM), IR is the intake rate (kg d-1) and BW is the body weight (kg). AF is the absorption fraction (dimensionless), i.e. the fraction of the ingested dose that is absorbed. Since the (ATSDR, 2003) reports that fluoride is absorbed in a range between 75 % and 100 % during the digestion process, these two limits where both assessed in two different scenarios.

The IR was estimated as follow:

$$\text{IR} = \frac{DCI \times FEC}{E} = \frac{(DCI\ kg{-}1) \times BW \times FEC}{E} \text{ (kg d}^{-1}\text{)}$$

where DCI is the Daily Calories Intake (kcal d^{-1}) that is given by the DCI kg^{-1} of BW multiplied by the BW, FEC is the Food Energy Contribution of each considered food item over the DCI. On the bases of the administered survey (Rizzu, et al., 2017) the FEC was estimated to be 26.3 % for maize, 0.2 % for tomato, 6.8 % for bean and 1.5 % for kale. E represents the energy of each food item (kcal kg^{-1}).

Since the BW occurs in both the numerator and the denominator, the equation was simplified in the following way:

$$EADDfood = \frac{C \times DCI\ kg - 1 \times BW \times FEC \times AF}{BW \times E} = \frac{C \times DCI\ kg - 1 \times FEC \times AF}{E}$$

The cumulative site-specific EADD was calculated by summing the contribution of the four crops:

EADDcumulative = EADDmaize + EADDbean + EADDtomato + EADDkale

Health risk assessment

In order to evaluate the individual risk deriving from the dietary fluoride exposure, the hazard quotient (HQ) associated with every food item was calculated for the three sites:

HQ = EADD/RfD (USEPA, 2001)

where RfD is the reference dose for humans associated with the 'no adverse effect level' (NOAEL). (USEPA, 2003) recommended a value of RfD for fluoride, which is 0.06 mg kg^{-1}.

The cumulative hazard index (HI), deriving from the crop-specific HQs was calculated as follows:

HI = HQmaize + HQbean + HQtomato + HQkale

Considering that IR depends on DCI kg^{-1}, and in turns, DCI kg^{-1} is related to the age, gender, weight and lifestyle of each individual, EADD and HI were reported by DCI kg^{-1} values. DCI kg^{-1} values for different stages of development, gender and lifestyle recommended by FAO (2001) were rearranged in graphs, in order to easily identify the values of EADD/HI per each category.

Statistical analyses

Soil soluble fluoride contents were compared to the limit for available fluoride in the soil established by EPA, FAO, and WHO of 16.44 mg kg^{-1} (Lakshmi, et al., 2017a, Limón-Pacheco, et al., 2018, Paul, et al., 2011) by using a one-tailed t-test. Data on fluoride partitioning in plant tissues were analysed according to a factorial ANOVA considering both locations and plant parts as random factors within each crop. Multiple comparisons of means related to fluoride partitioning in plant tissues were then performed using a Tukey-test. The ANOVA was performed with the R software (R Core Team, 2014).

Results

Soil water-soluble fluoride

Soil water-soluble fluoride concentrations in the three study areas ranged between 4 and 8 times above the limit for available fluoride in the soil (16.4 mg kg^{-1}) established by EPA, FAO, and WHO (Lakshmi, et al., 2017a, Limón-Pacheco, et al., 2018, Paul, et al., 2011) (Table 10).

Table 10 - Soil water-soluble fluoride content (mean ± standard error, n=12) in the three study sites

Study site	Soil water-soluble F^- (WSF^-, mg kg^{-1})	WSF^-/ULF§	Significance of t test
Momela	63.7 ± 2.5	3.9	***
Uwiro	133.1 ± 9.0	8.1	***
Olkung'wado	129.6 ± 52.7	7.9	*

§ WSF^-/ULF = Water-Soluble F^-/ limit of available fluoride in the soil

* =significant for $P \leq 0.05$; *** =significant for $P \leq 0.001$.

Fluoride uptake and partitioning in plants

Fluoride concentrations in different plant parts (leaves, stem, roots and grains/fruits) of the four studied crops are reported in Table 11.

Concerning maize and tomato, no significant interaction between sites and plant parts was found in terms of concentration of fluoride in the different plant parts (roots, leaves, stem and grains/fruits) following the same accumulation pattern in all the study sites: roots > leaves > stem > grains/fruits. In tomato, significant differences between sites were also observed with the highest fluoride plant accumulation in Olkung'wado and the lowest in Momela. A significant site x plant part interaction was observed for the fluoride plant uptake in bean. In Momela, the accumulation of fluoride was higher in roots compared to stem and seeds. In Olkung'wado, on the contrary, seeds accumulated

more fluoride than roots and stem showed an intermediate value between roots and seeds. In Uwiro, no significant differences were observed in terms of fluoride accumulation in the three plant parts. In kale a significant site x plant part interaction was observed for the fluoride concentration in plants. In Momela and Uwiro the bioaccumulation of fluoride in kale plant tissues was highest in roots followed by leaves and stems that showed similar fluoride concentration. In Olkung'wado the fluoride accumulation trend was the following: roots > leaves >stems.

Table 11 - Fluoride concentration in maize, tomato, bean and kale plant tissues at the physiological maturity stage of development (mg kg^{-1} of dry matter) and partitioning into plant parts for the three study sites.

Crop	Plant part	Momela	Uwiro	Olkung'wado	*Mean*
Maize	Grains	8.9	7.8	7.4	*8.0 d*
	Leaves	25.8	24.3	23.8	*24.6 b*
	Stem	15.0	10.6	16.0	*13.9 c*
	Roots	37.3	38.6	38.1	*38.0 a*
	Mean	*21.7 A*	*20.3 A*	*21.31 A*	*21.1*
Tomato	Fruits	12.9	11.4	10.3	*11.4 c*
	Leaves	33.8	37.3	40.3	*37.6 a*
	Stem	10.4	17.1	25.6	*18.6 b*
	Roots	29.4	35.1	35.4	*33.8 a*
	Mean	*21.6 B*	*25.3 AB*	*27.9 A*	*25.3*
Bean	Seeds	10.4 Ab	11.3 Aa	12.7 Aa	*11.3*
	Stem	4.0 Ab	8.5 Aa	6.9 Aab	*6.4*
	Roots	23.3 Aa	16.3Aa	3.4 Bb	*15.7*
	Mean	*12.6*	*12.0*	*7.7*	*11.1*
Kale	Leaves	9.0 Bb	12.7 Bb	20.9 Ab	*14.2*
	Stem	7.5Ab	5.0 Ab	3.6 Ac	*5.3*
	Roots	23.6 Ba	33.7 Aa	37.9 Aa	*31.7*
	Mean	13.4	17.1	20.8	*17.1*

Means followed by the same letter are not statistically different at $P \leq 0.05$; capital letters refer to comparisons among sites, lower-case letters to plant parts.

Bioconcentration (BCF) and translocation factors (TF)

In order to evaluate the fluoride concentration in the aerial biomass tissues (ESS) or edible part (EP) as compared with the water-soluble fluoride concentration in soil, the BCF was calculated for the studied crops.

The highest value of the BCF in the ESS was recorded in tomato, in Olkung'wado, while the lowest one in maize, in Uwiro. Regarding the BCF of EP the highest value was observed in Olkung'wado in the bean crop, and the lowest in maize, in Uwiro (Table 12).

The capability of plants to transfer fluoride from roots to the whole shoots or to the edible parts was evaluated through the calculation of the TF. The highest TF of EP was observed for bean (3.72) and the lowest for maize (0.19), both in the Olkung'wado experimental site while for the TF of ESS, the highest and the lowest values were 2.56 for bean and 0.23 for maize respectively, in Olkung'wado and Uwiro sites (Table 12).

Table 12 - Bioconcentration (BCF) and translocation (TF) factors calculated for the investigated crops in the three study areas of Momela, Uwiro and Olkung'wado.

Study site	Crop	BCF ESS *	BCF EP **	TF ESS §	TF EP §§
Momela	Tomato	0.30	0.18	0.77	0.48
	Bean	0.13	0.17	0.39	0.52
	Kale	0.20	0.15	0.52	0.40
	Maize	0.22	0.14	0.38	0.24
Uwiro	Tomato	0.27	0.13	0.68	0.33
	Bean	0.07	0.07	0.74	0.83
	Kale	0.11	0.08	0.52	0.40
	Maize	0.07	0.06	0.23	0.20
Olkung'wado	Tomato	0.45	0.19	0.66	0.29
	Bean	0.28	0.42	2.56	3.72
	Kale	0.24	0.16	0.35	0.57
	Maize	0.38	0.31	0.29	0.19

* BCF ESS (Bioconcentration factor considering entire shoot system) = F in entire shoot system / F in soil

** BCF EP (Bioconcentration factor considering the edible part = F in the edible part / F in soil

§ TF ESS = F in entire shoot systems / F in roots

§§ TF EP = F in edible part / F in roots

Exposure dose and health risk assessment

Cumulative fluoride exposure dose (EADD) and hazard index (HI) for each study area at two hypothetical limit levels of fluoride absorption (AF) in the human body (75% and 100%) are shown in Figure 2. The highest values were observed for a daily energy intake of 90 kcal d^{-1} kg^{-1} of BW, that is the typical energy consumptions for kids at very earlier stages, while the lowest were associated with values of 24 kcal d^{-1} kg^{-1} of BW, characteristics of elder people with a sedentary lifestyle (Figure 3).

Considering a fluoride absorption of 75%, the EADD values ranged from 0.026 to 0.098 in Momela, from 0.027 to 0.102 in Uwiro and from 0.033 to 0.124 mg F d^{-1} kg^{-1} of BW in Okung'wado. With the 100% F absorption scenario, the values of EADD ranged from 0.035 to 0.130 in Momela, from 0.036 to 0.136 and from 0.044 to 0.165 mg F d^{-1} kg^{-1} of BW in Okung'wado.

Concerning HI, minimum values were 0.43, 0.45, 0.55 (AF 75%) and 0.58, 0.60, 0.73 (AF 100%) in Momela, Uwiro and Olkung'wado respectively. Maximum values were 1.63, 1.70, 2.06 (AF 75%) and 2.17, 2.27, 2.75 (AF 100%).

The contribution of single hazard quotients (HQs) for each crop to the cumulative HI for each study area are reported in Figure 3. In all the study sites, the main contribution to the HI was given by maize crop, apart from Olkung'wado, in which kale played a major role.

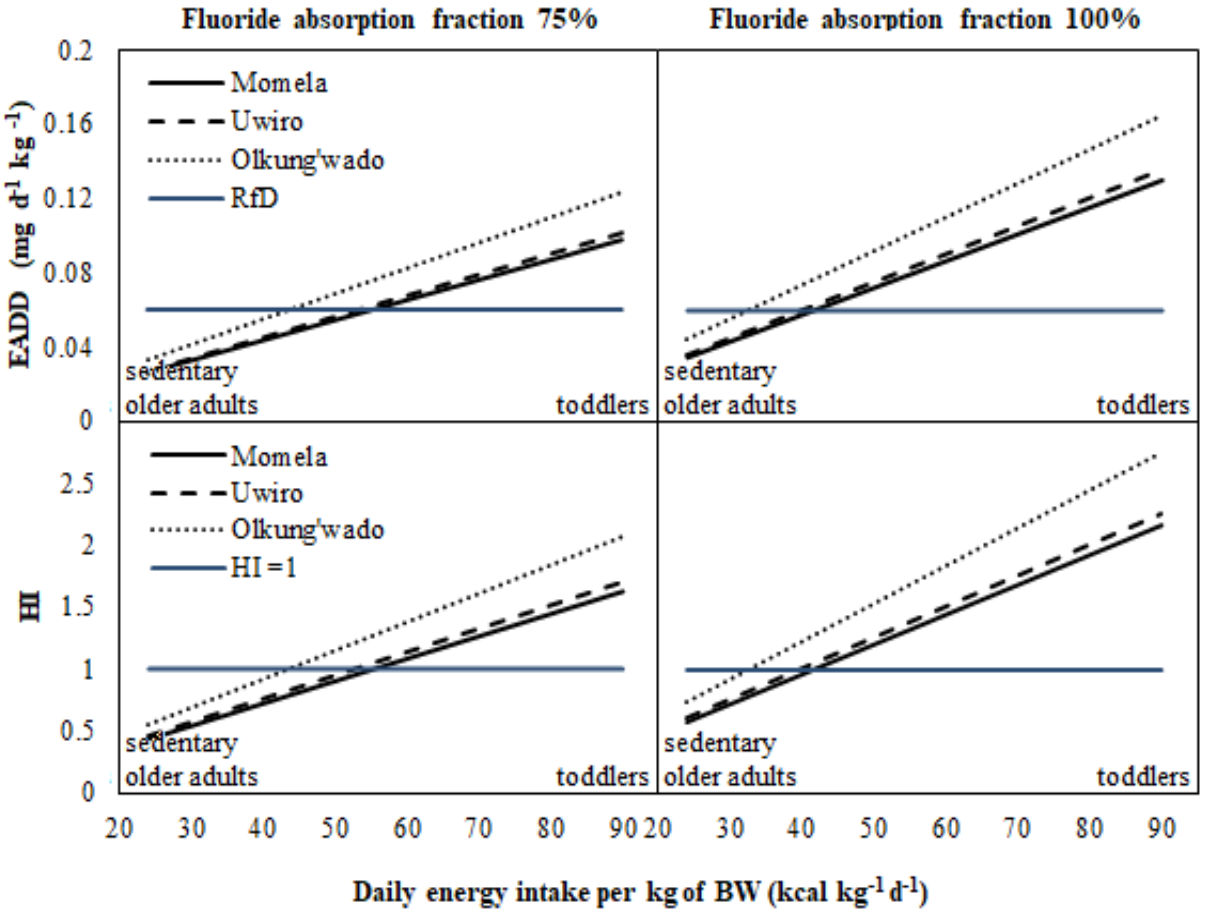

Figure 2 - Cumulative EADD and HI, related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado, at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human body (75% and 100%) were assessed. RfD is the reference dose for humans associated with the 'no adverse effect level'. HI > 1 is associated to increasing risks for human health (USEPA, 2001).

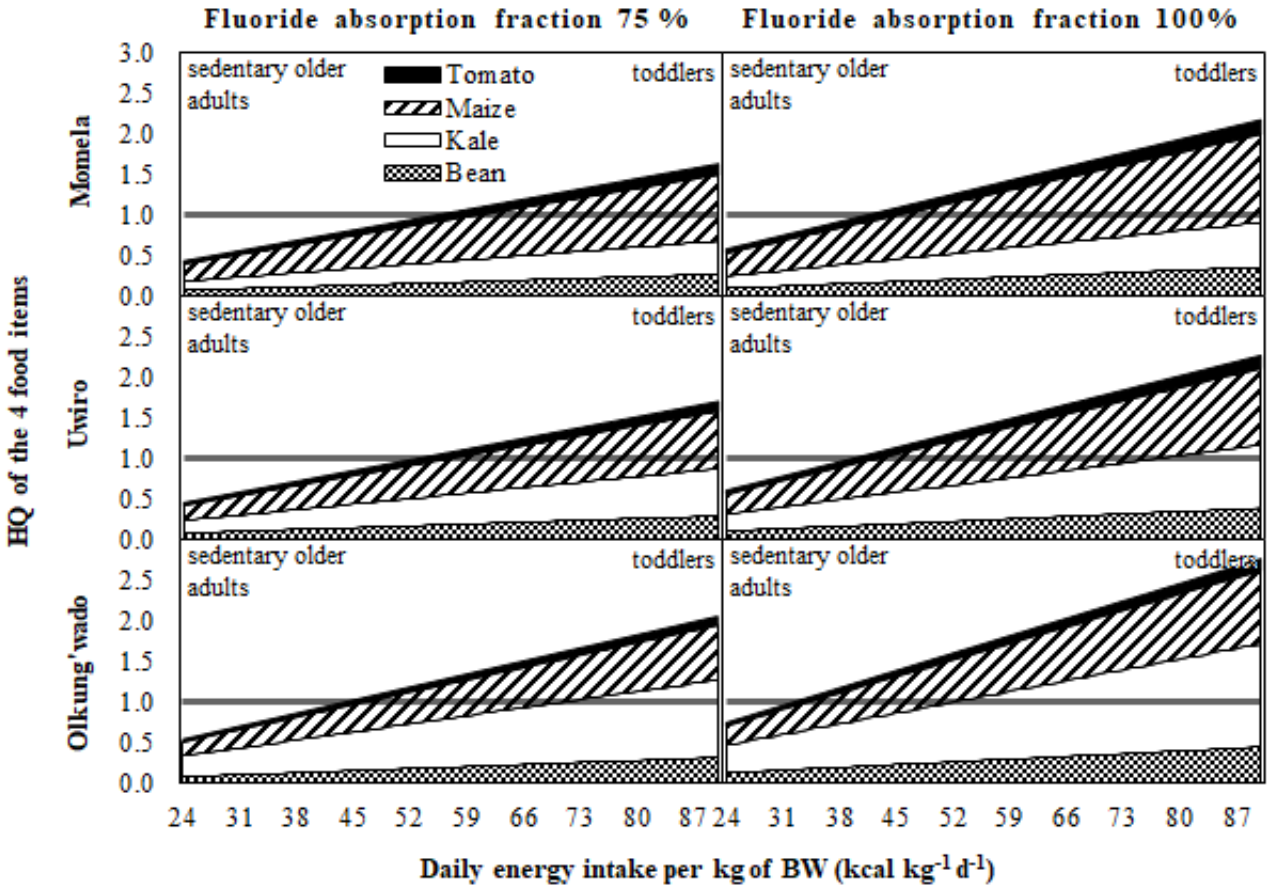

Figure 3 - Contribution of each crop to the cumulative HI related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human body (75% and 100%) were assessed. HI > 1 is associated to increasing risks for human health (USEPA, 2001).

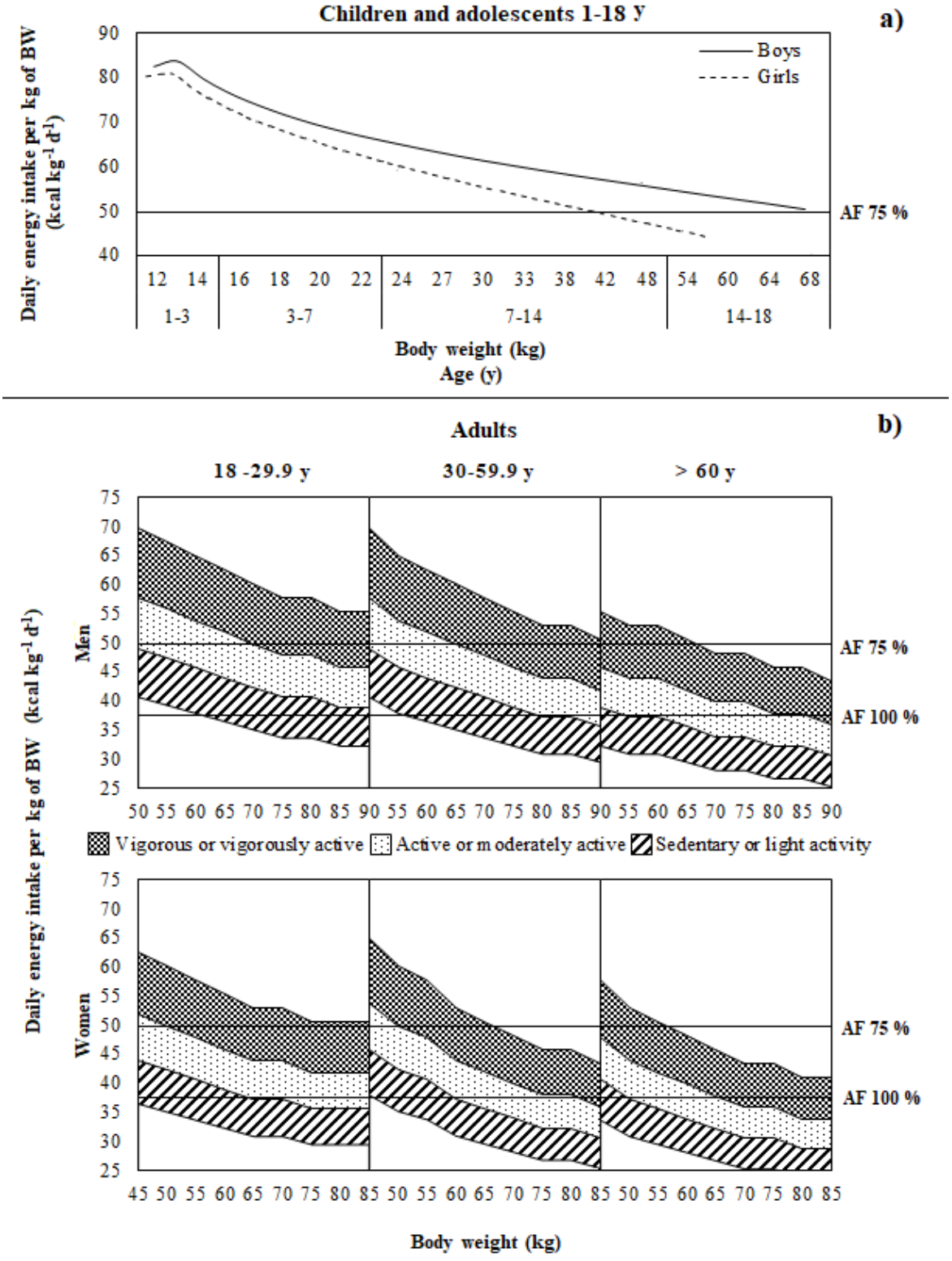

Figure 4 - Daily energy intake per kg of body weight by different ages, weights and lifestyle categories: a) children and adolescents, b) adults (rearranged from FAO (2001)). All the categories falling above the AF 75 % and AF 100 % lines correspond to the population groups with HI > 1 in the AF 75 % and AF 100 % scenarios respectively.

Discussion

Soil water-soluble fluoride

The range of soil water-soluble fluoride detected is consistent with values found by Dagnaw et al. (2017) in a study on the fluoride content of leafy vegetables in the Rift valley or elsewhere. These values are always significantly higher than the threshold of 16.4 mg kg^{-1} in soil of available fluoride established by EPA, FAO, and WHO as cited by (Lakshmi, et al., 2017a, Limón-Pacheco, et al., 2018, Paul, et al., 2011).

Soil water-soluble fluoride is highly related to the soil type: factors as pH, clay minerals, organic matter and concentration of P and Ca are the main drivers of fluoride solubility in soil. Furthermore, in sodic soils, a high exchangeable Na increases fluoride solubility (Kabata-Pendias, 2011). In the present study, high soil water-soluble fluoride values can be explained by high pH and poor organic matter content, clay minerals and exchangeable Ca content. At high pH, in fact, an increase of disadvantageous electrostatic potential limits the capability of the soil to retain fluoride, increasing F^- concentration in the soil solution (Choudhary, et al., 2019). In addition, sandy soils, as those of the study sites, have the lower capability of F-retention than fine-textured soils because clay minerals containing Fe and Al can form very stable bonds with fluoride (Edmunds and Smedley, 2013, Omueti and Jones, 1977). Calcium scarcity in soil is another factor related to the mobility of the F element since Ca can bound F in low soluble species as CaF_2. On the contrary, a high presence of Na is related to high F solubility since NaF is one of the most mobile compounds (Kabata-Pendias, 2011, Kabata-Pendias and Szteke, 2015).

Fluoride concentration in plant tissues, partitioning, bioconcentration (BCF) and translocation factors (TF)

Similar to what was found by other authors in various plant species, roots were, in the majority of cases, the organs were F accumulated more (Baunthiyal and Ranghar, 2014, Baunthiyal and Ranghar, 2015, Chakrabarti, et al., 2013b, Lakshmi, et al., 2017a, Takmaz-Nisancioglu and Davison, 1988), except for tomato in which roots and leaves fluoride content were not significantly different and bean in Olkung'wado, where roots had lower values of fluoride with respect to seeds. This peculiarity could be explained by the saline-sodic nature of the soil of this area, which may have induced bean roots stunting (Pessarakli, 1999), causing an imbalance both in biomass and fluoride partitioning in the different organs of the plant. In this regard, Kafkafi (1991) reported that a reduction in bean root elongation, related to saline soils, was a factor preventing the quantity of ions that reach the roots by diffusion, in order to limit their plant uptake just at the required quantities.

In all the examined crops, the second plant organs for fluoride concentration level were the leaves. This trend was observed also by Chakrabarti et al. (2013b) and Gupta and Banerjee (2009) on rice, Singh et al. (1995) on lady's finger and Yepu et al. (2017) on wheat. Ahmad et al. (2015) and Yadav et al. (2018) on mustard and Gautam and Bhardwaj (2010a) on barley found instead that grains accumulated more fluoride than leaves.

For all the considered species in each site, BCF values were < 1, indicating that none of them behaves as hyperaccumulator of fluoride in the study area. In addition, it was observed that an increase in soil water-soluble fluoride corresponds to a general decrease in BCF values as reported by Zhang et al. (2018). This trend could be interpreted as a defence mechanism countering the high fluoride toxicity. In addition, TF values were always less than one in all crops, illustrating their lower accumulation in shoot systems or edible parts than in roots. The only exception was represented by bean in

Olkung'wado since its peculiar minor accumulation in roots already described. In general, there are only a few studies regarding fluoride partitioning into different plant parts and even less for the species investigated in the present study. Nagaraju et al. (2017) detected higher fluoride concentration in bean stems compared to that observed in the present work, despite a lower fluoride concentration in soil, hence the BCF calculated on the basis of this data would be greater than the values obtained in the current study for beans. Fluoride concentration in roots and grains were not reported by Nagaraju et al. (2017) hence it was not possible to compare our results with TF values from this study. The higher accumulation of fluoride in roots was confirmed by a study of Szostek and Ciećkob (2014) who found that roots had on average 6-fold more fluoride compared to aerial biomass. From their data, it was possible to calculate the TF in ESS, which ranged from 0.11 to 0.26, while in our study TF in ESS ranged from 0.23 to 0.38. Instead, TF EP values could not be calculated from the same study, since no data were reported about the fluoride concentration in edible parts.

The analyses derived from the data collected on tomato by Bhattacharya et al (2017), revealed an average value of BCF in EP of 0.05, which was about 3 times lower than that observed in our case (0.17). With respect to TF in EP, Lakshmi et al. (2017a) data revealed a mean value of 0.54, while our findings indicate an average TF of 0.37.

Among the examined crops, kale showed a higher fluoride concentration in edible parts on average, given that the comestible organs are the leaves. The values of BCF in EP in kale was 0.13 while from the data reported by Dagnaw et al. (2017) we calculated a BCF value of 0.24.

The differences between our study and the other studies cited above are not surprising, considering that BCF values can vary among the same vegetable species depending on all the factors that influence plant development such as the soil features and growth rate (Swartjes, et al., 2007).

Exposure dose and health risk assessment

The results of EADD and HI, calculated for the three different areas of Momela, Uwiro and Olkung'wado, chosen as representatives for the study area, highlight that the main consumed crops by local populations give a significant contribution to the individual daily fluoride exposure and to the associated HI.

When a value of HI < 1 is found, there is no significant risk of non-carcinogenic effects while for HI values > 1, possible non-carcinogenic effects may occur, with increasing probability at higher HI values (USEPA, 2001). Children in very early stages are the most vulnerable to fluoride diseases from chronic exposure since, due to their high daily energy requirement per kg of body weight; they are more susceptible to reach the highest daily fluoride intake in proportion to their weight. Among adolescents, only girls from 14 to 18 years old fall in the HI < 1 range in the AF 75 % scenario.

Concerning adult population groups considered in this study, just a few categories fall in the HI < 1 range with an AF 100 % hypothesis, sedentary adults and, among women, also part of the moderately active group. In the AF 75 % scenario instead, most of the categories fall in the HI < 1 range, apart from very active lifestyle men and women and moderately active young men.

Conclusions

This study highlighted that water-soluble fluoride content in agricultural soils of Ngarenanyuki area was greater than the limit of 16.4 mg kg^{-1} as established by EPA, FAO, and WHO Joint Standards, with an average of 63.7 ± 2.5, 133.1 ± 9.0, 129.6 ± 52.7 ppm in Momela, Uwiro and Olkung'wado subareas respectively.

Despite all the studied crops showed BCF < 1 values, indicating that none of them is hyperaccumulator in the study area, fluoride movement from contaminated soils to food crops was reflected in a substantial accumulation of fluoride in plants edible parts. Average concentrations of

14.2, 11.4, 11.3 and 8.0 ppm were observed for kale, tomato, bean and maize respectively, demonstrating that the considered food items, that are among the most consumed in the rural area under study, substantially contribute to fluoride-correlated diseases, especially in earlier ages, also without considering the contribution of the drinking water. For children and adolescents, a high risk of non-carcinogenic effects from fluoride exposure was highlighted, for both AF 75 % and AF 100 % hypothesis, whereas regarding the adult population groups a big difference emerged among AF 75 and AF 100 % scenarios, with a wider range of under-risk people in the latter case.

The results obtained in this study are only referred to crops grown in the EARV in the wet season, thus, further experiments are needed to assess the bioaccumulation of fluoride in food crops during the dry season, when F-rich irrigation water is employed to satisfy crop water requirements. Furthermore, the soil soluble fluoride dynamics and its relation with the contaminated irrigation waters are worth further investigations. Crops whose edible organs are roots, tubers and bulbs (e.g. carrots, potatoes, onion and turnip) are also worth to be investigated since they could possibly accumulate a higher amount of fluoride in edible parts.

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Chapter III

Impact of high-fluoride water on the growth and F-bioaccumulation patterns of maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill.) and bean (*Phaseolus vulgaris* L.)

Impact of high-fluoride water on the growth and F-bioaccumulation patterns of maize (Zea *mays* L.), tomato (*Lycopersicon esculentum* Mill.) and bean (*Phaseolus vulgaris* L.)

Abstract

The chronic exposure to excessive fluoride doses can lead to a series of severe diseases, known under the name of endemic fluorosis, that affect numerous areas of Asia, Africa and America. The association between those illnesses and the environmental geogenic fluoride pollution is well documented. One of the most fluoride-contaminated areas is located in the East African Rift Valley, where groundwaters and surface waters recorded some of the highest concentrations of fluoride ever observed. In addition to the consumption of drinking waters exceeding the WHO limit of 1.5 mg F^- L^{-1}, the ingestion of contaminated food may represent another important source of fluoride exposure. A greenhouse pot experiment was conducted with the aim to assess the impacts of increasing fluoride levels in the irrigation water (0, 50, 150, 400, 800, 1600 mg F^- L^{-1}) on the productivity and the fluoride accumulation of some of the most cultivated crops of the East African Rift Valley.

Fluoride concentration in edible parts of maize, tomato and bean ranged between 7.9-12.4, 8.0-15.4 and 7.1-9.5 mg F^- kg^{-1} DM, respectively. The protracted use of high-fluoride contaminated water along the crop cycle considerably affected the quality of the soil leading to a significant rise of its water-soluble fluoride concentration. Moreover, for all the studied species, the fluoride accumulation in crop residues increased with the increased fluoride concentration in the irrigation, representing an indirect risk for human health. In the rural areas of the East African Rift Valley, in fact, crops residues of maize and bean are mainly used for feeding livestock so that fluoride may enter the food chain. In the case of tomato, instead, residues that are left in the field or mulched can contribute to increase the fluoride content of the top soil, exacerbating the fluoride contamination.

Keywords: Fluoride, water pollution, maize, tomato, bean, Bioconcentration Factor.

Introduction

Environmental geogenic fluoride contamination is a worldwide spread issue affecting several areas of Asia, Africa and America (Ali, et al., 2016, Vithanage and Bhattacharya, 2015). Although on a local scale, industrial and agricultural activities (e.g. aluminium smelting, excessive use of phosphate fertilizers etc.) can represent a significant source of fluoride pollution, in numerous regions of India, China, Sri Lanka, Pakistan, Argentina, Mexico, south and east Africa and many others, fluoride contamination of the environment is of natural origin, mainly resulting from geological processes (Amini, et al., 2008, Edmunds and Smedley, 2013, Kimambo, et al., 2019, Vithanage and Bhattacharya, 2015).

The geogenic occurrence of fluoride is frequently related to volcanism phenomena, as in the case of the East African Rift Valley, where the rift geological instability has a serious impact on the quality of soil, water and food (Davies, 2008). In these areas, in fact, groundwater, alkaline lakes, rivers and hot springs recorded some of the highest concentrations of fluoride ever observed. Among the various processes that lead to the anomalous environmental fluoride incidence, there is the development, and subsequent weathering, of hyper-alkaline fluoride-rich volcanic rocks (Edmunds and Smedley, 2013, Malago, et al., 2017). From soil and water, fluoride is introduced into the ecosystems reaching humans through the consumption of contaminated drinking water, food products, and to some extent, through the air (Vithanage and Bhattacharya, 2015).

A series of severe diseases, named as endemic fluorosis, are well known to be related with the chronic exposure to excessive fluoride doses in contaminated areas. Dental effects, such as enamel mottling and pitting, are associated mainly with the moderate long-term ingestion since early ages, whereas the prolonged intake of larger amounts can seriously affect the skeletal system. Symptoms can include joint tightness and pain, ligaments calcification and structural bone alterations (WHO, 1999, 2011). Moreover, in recent years, research has increasingly focused on toxic effects of fluoride on non-

skeletal tissues. Nervous, cardiovascular and reproductive systems, as well as kidney, liver, pancreas and thyroid, are also prone to be affected by excessive fluoride intakes (Wei, et al., 2019). A long-term consumption of drinking water exceeding the WHO guideline limit (1.5 mg $F^- L^{-1}$) is considered the main source of fluoride exposure, nevertheless the ingestion of contaminated food can represent another important route for fluoride entry in the human organism (Brahman, et al., 2014, WHO, 2011). The accumulation of fluoride in food and feed crops cultivated in polluted areas, and also the effects that this element can have on crop productivity, are related to the plant species itself and the bioavailability of fluoride in the soil solution (Bhattacharya, et al., 2017, Okibe, et al., 2010).

In spite of some of the main food crops have been investigated by the literature in this respect (Bustingorri and Lavado, 2014, Chakrabarti, et al., 2013b, Wang, et al., 2012), there are very few studies that consider species like maize, bean or tomato that represent important cultivated and consumed crops in the contaminated areas of the East African Rift Valley. Thus, the objectives of this study were to assess the impacts of increasing fluoride levels in the irrigation water on: (i) the productivity of some of the main crops grown in the East African Rift Valley (maize, bean and tomato) and, in particular in the area of Ngarenanyuki ward in Arumeru District, Arusha Region in North Tanzania; (ii) the concentration of bioavailable fluoride in the soil for plants (soil water-soluble fluoride) (iii) the fluoride plant uptake and its partitioning among the different organs of the plant (edible parts for human consumption such as fruits or seeds and animal feeding parts e.g. stem and leaves) in order to calculate their respective bioconcentration factors.

Materials and methods

Crop selection

The selection of the most interesting crops to be investigated was made according to the outcomes of a survey on dietary habits and "business as usual" agricultural practices at household-scale submitted to the local farmers in the case study areas of the East African Rift Valley (Rizzu, et al., 2017).

Based on the survey, maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill.) and bean (*Phaseolus vulgaris* L.) were identified as the main cultivated and self-consumed crops in the area of of Ngarenanyuki ward in Arumeru District, Arusha Region in North Tanzania. As regards the fate of crops harvest residues, the survey highlighted that, in the case of maize, 76% of residues were destined to domesticated animal feeding and 17% left in the field or mulched. For bean, these percentages became 69% and 25%, respectively. In the case of tomato, at the opposite, the percentage of residues left in the field or mulched (73%) was paramount compared to that given to the animals (20%).

Experimental design

From July to December 2017 a pot experiment was conducted in the experimental farm greenhouse of the Agricultural Science Department of the University of Sassari, Italy (40°46'32.1"N 8°29'16.8"E). On June 2017, seeds of maize, tomato and bean were germinated in a growth chamber maintained at 24°C and 80% RH with a 18 h photoperiod. After 10 days, seedlings were moved in the greenhouse and after other 7 days one plant was transplanted into each pot wetted to field capacity. For all the three crops, materials were provided by the Nelson Mandela African Institute of Science and Technology in Arusha (Tanzania) among the most cultivated cultivars/ecotypes (*Zea mays* L. cv.

Situka, *Phaseolus vulgaris* L. cv. Jesca, *Lycopersicon esculentum* Mill. cv. Rio Grande) in the case study area of Ngarenanyuki ward in Arumeru District, Arusha Region in North Tanzania.

For tomato and bean 18 L plastic pots were filled with 23 kg of substrate, while in the case of maize 40 kg of substrate was placed in 45 L pots. The substrate was composed of 20% of washed sand and 80% of the top horizon of a sandy clay loam soil taken from the surface layer of arable land in the countryside of San Sebastiano, Ploaghe, Italy (40°39'50"N, 8°43'1"E). The soil was chosen to be as similar as possible in terms of texture to that of the study area in Tanzania. Once collected, the soil was air-dried and impurities such stones and roots were removed. Soil chemical and physical properties and references to the methods used for their determination are reported in Table 13.

Table 13 - Characteristics of the soil used for the pot soil substrate.

Soil trait	Value	Reference of the analytical method
Sand (%)	61.2	Astm D422 and UNI CEN ISO/TS 17892-4*
Silt (%)	16.8	
Clay (%)	22.0	
pH (1:2) (H20)	7.5	ISO 10390
EC (1:2) (μS cm^{-1}) 25 °C	225	ISO 11265
Organic C %	2.0	ISO 10694**
Total N %	0.2	Method XIV.1 G.U. 248 21/10/1999**
C/N	11	
Exchangeable Ca^{2+} (meq 100 g^{-1})	13.0	ISO 11260****
Exchangeable Mg^{2+} (meq 100 g^{-1})	1.3	
Exchangeable Na^{+} (meq 100 g^{-1})	0.4	
Exchangeable K^{+} (meq 100 g^{-1})	0.2	
Exchangeable base cations (EBC) (meq 100 g^{-1})	14.9	
CEC (meq 100 g^{-1})	15	ISO 11260
Base saturation (BS) (%)	100	

*GSA - GRAIN SIZE ANALYSER (Gibertini, Milano (MI), Italy)
** CHN628 Series Carbon, Hydrogen, Nitrogen Elemental Determinator (LECO, St. Joseph (MI), U.S.A)
*** Cary 60 UV-VisSpectrophotometer (Agilent Technologies, Selangor, Malaysia)
****AAnalyst 200 Atomic Absorption Spectrometer (PerkinElmer, Shelton (CT), U.S.A)

The contaminated solutions were prepared by adding different concentrations of sodium fluoride (NaF) to deionized water (0, 50, 150, 400, 800, 1600 mg $F^- L^{-1}$) and irrigation was conducted twice a week in order to keep a good plant water status. Pots were weighed twice a week and the amount of irrigation water was calculated to rise again the soil water at the field capacity. Each treatment was replicated three times for a total of eighteen pots per species arranged in a completely randomized design. In order to prevent nutritional deficits, a fertilization plan based on the specific needs of each crop was adopted (Table 14).

Table 14 - Fertilizer doses applied to the three investigated crops at different development stages. Values are expressed in terms of g $plant^{-1}$.

Crop	Development stage	N-P-K Ratio	Dose (g $plant^{-1}$)	N (g $plant^{-1}$)	P (g $plant^{-1}$)	K (g $plant^{-1}$)
Bean	Pre-planting	8-20-24	5	0.4	1	1.2
Tomato	Pre-planting	20-5-10	30	6	1.5	1
Maize	Pre-planting	8-20-24	21	1.7	4.2	5.0
Maize	Stem elongation	26	6.4	1.7	-	-
Maize	Stem elongation	26	6.4	1.7	-	-

Data on morphological traits (e.g. plant height, number of leaves, buds, fruits/pods etc.) were collected at the maturity stage.

Sample collection and preparation

At the maturity stage for each crop (85, 110 and 160 days from transplanting for bean, maize and tomato respectively), plants were harvested and partitioned into stems, leaves, pods and grains/fruits. In the case of maize and tomato stem and leaves were kept together. Since one month before the final destructive sampling, tomato fruits were continuously harvested at half-ripe stage for a total of eleven sampling dates. Plant parts were weighted fresh and after oven drying (72 hours at 60ºC) they were ground to a fine homogeneous powder using a Retsch ZM 200 (Retsch, Haan, Germany) ($\varphi < 40$ μm).

Two soil samples per pot were collected and bulked together. Samples were oven-dried at 105 °C for 72 h, ground and sieved (2 mm mesh sieve) to get homogenized representatives samples.

Chemical analyses

For total F determination in plant tissues, powdered vegetable samples (0.2 g) were digested with 2 ml of nitric acid 67%, hydrogen peroxide at 30% (3 ml) and deionised water (5 ml), in a microwave digestion unit (Milestone Ethos Easy, Milestone s.r.l, Sorisole (BG), Italy). In order to avoid losses of F in the form of HF, the vapour pressure of nitric acid was reduced immersing the still closed vessels in a refrigerated bath (-30 °C) for 30 min. The neutralization of digest solutions with aqueous NaOH (8M) was carried out directly inside the vessels immediately after their opening. This procedure allowed to rapidly stop the leakage of nitrous fumes and the consequent loss of analyte.

For soil water-soluble fluoride extraction, 2 g of sieved soil were shaken in 40 ml of deionized water (ratio 1:20) for 16 - 18. The extraction fluid/sample mixture was centrifuged and a 10 ml aliquot from the supernatant was removed for the estimation of F.

Both F concentration of the plant samples digest solution and soil samples water extract were determined potentiometrically using a fluoride ion-selective electrode (ISE - Thermo Scientific Orion 9609BNWP). In order to adjust ionic strength, buffer pH to 5.0-5.5, and break up metal-fluoride complexes, standards for the instrument calibration and samples were mixed 1:9 with a total ionic strength adjustment buffer (TISAB III) one hour prior to the determination.

Data analyses

Bioconcentration Factor (BCF)

Translocation of fluoride between soil and plant can be assessed through an index defined as Bio-Concentration Factor (BCF) which is obtained by dividing the F^- concentration in the plant (or specific organ) by the soil water-soluble fluoride concentration (Bustingorri and Lavado, 2014):

$$BCF = \frac{F\ concentration\ in\ plant\ part\ or\ entire\ shoot\ system\ (\frac{mg\ F^-}{kg\ of\ DM})}{Water\ soluble\ F^-\ concentration\ in\ soil\ (\frac{mg\ F^-}{kg\ of\ soil})}$$

Statistical analyses

Multiple comparisons of means related to biometric variables and soil water-soluble fluoride were performed using the Fisher LSD Method (95% Confidence) following a one-way ANOVA with the levels of fluoride in the irrigation water as treatment. Multiple comparisons of means related to fluoride analyses and BCFs were performed using the Fisher LSD Method (95% Confidence) following a two-factor factorial (treatment x plant organ) ANOVA with three replicates performed with the Minitab 17 Statistical Software (2010).

The hypotheses whether the soil soluble F concentration was significantly higher or lower than the limit of 16.4 mg kg^{-1} in soil (available fluoride) established by EPA, FAO, and WHO as cited by Lakshmi et al. (2017a), Limón-Pacheco et al. (2018), Paul et al. (2011) were tested using a one-tailed t-test.

Results

Effects of fluoride on plants survival rate and yields and yield components

For all the three investigated crops, a survival rate of 100% was observed when plants where irrigated with fluoride contaminated water ranging from 0 to 800 mg F^- L^{-1}. When the level of fluoride rose to 1600 mg F^- L^{-1} the survival rate was 100% for maize and 0% for tomato and bean.

Concerning maize yield and yield components, the production of grains, the number of kernels and the thousand kernel weight were no affected by the irrigation with water at different F^- concentration. As regards crop residues, the dry matter production of maize stem and leaves reflected a similar trend to that of plant height at the maturity stage. These two variables, in fact, seemed to be positively influenced by increasing F^- concentration in irrigation water, reaching the highest values for plant irrigated with 400 and 800 mg F^- L^{-1} and subsequently decreasing until no significant differences between control plants and those treated with 1600 mg F^- L^{-1} (Table 15).

Regarding the total production of tomato fruits, no differences were found between treatments. In fact, the decreasing number of fruits per plant with increasing F^- treatments, was counterbalanced by a, even not significant, higher fruit weight and calibre. Fruits with a calibre smaller than 20 mm were excluded from the estimation of the marketable production, which, however, was not different between treatments. The biomass of crop residues (sum of stem and leaves) showed the highest value in the control treatment and gradually decreased with increasing F^- concentration. The lowest value was observed for plants irrigated with 800 mg F^- L^{-1} that recorded a biomass reduction of around 68% compared to the control (Table 16).

As regards to the bean, an increase of around 177% in seed production was observed for plant irrigated with 50 mg F^- L^{-1} compared to control. The plants treated with 150 and 400 mg F^- L^{-1} showed intermediate values between control plants and those treated with 50 mg F^- L^{-1}. The lowest production

was observed when irrigation with 800 mg F^- L^{-1} was applied but without significant differences with respect to the control. The production of stem and leaves was the highest in plants irrigated with 50 and 150 mg F^- L^{-1} recording almost doubled values compared to those treated with 0 and 800 mg F^- L^{-1} while the 400 mg F^- L^{-1} treatment showed an intermediate production (Table 17).

Table 15 - Morphological features at the maturity stage, yields and yield components of maize crop irrigated with water at different levels of F^- concentration (0, 50, 150, 400, 800 and 1600 mg F^- L^{-1}).

Crop	Variable	Levels of F^- concentration in irrigation water (mg L^{-1})						
		0	50	150	400	800	1600	Mean
Maize	Kernels production (g of DM $plant^{-1}$)	26.4 a	38.7 a	47.4 a	37.1 a	33.0 a	24.5 a	34.5
	Number of kernels (n $plant^{-1}$)	143 a	176 a	245 a	185 a	161 a	164 a	179
	Thousand kernels weight (g)	175 a	221 a	192 a	201 a	212 a	153 a	192
	Stem and leaves production (g of DM $plant^{-1}$)	72.9 c	92.2 bc	105.9 abc	130.5 a	112.3 ab	77.6 c	98.6
	Plant height (cm)	194 c	211 bc	232 ab	246 a	221 abc	200 c	217
	Number of leaves (n $plant^{-1}$)	17 a	19 a	19 a	19 a	17 a	17 a	18

Means followed by the same letter are not significant different for $P \leq 0.05$.

Table 16 - Morphological features at the maturity stage, yields and yield components of tomato crop irrigated with water at different levels of F^- concentration (0, 50, 150, 400 and 800 mg F^- L^{-1}).

Crop	Variable	Levels of F^- concentration in irrigation water (mg L^{-1})					
		0	50	150	400	800	Mean
Tomato	Total production: fruits (g of DM $plant^{-1}$)	24.6 a	23.8 a	21.2 a	22.5 a	16.9 a	21.8
	Number of fruits (n $plant^{-1}$)	24 a	20 b	17 bc	16 c	14 c	18
	Weight of a single fruit (g of DM $fruit^{-1}$)	1.0 a	1.2 a	1.3 a	1.4 a	1.2 a	1.2
	Fruit calibre (mm)	21 a	25 a	29 a	30 a	28 a	27
	Marketable production: fruits > 20 mm (g of DM $plant^{-1}$)	17.3 a	20.7 a	20.2 a	21.6 a	15.7 a	19.1
	Stem and leaves (g of DM $plant^{-1}$)	39.9 a	22.6 b	19.3 bc	16.3 bc	12.8 c	22.2
	Plant height (cm)	72 abc	80 a	74 ab	72 abc	66 c	73
	Number of leaves (n $plant^{-1}$)	47 a	37 ab	32 b	26 b	24 b	33

Means followed by the same letter are not significant different for P≤0.05.

Table 17 - Morphological features at the maturity stage, yields and yield components of bean crop irrigated with water at different levels of F^- concentration (0, 50, 150, 400 and 800 mg F^- L^{-1}).

Crop	Variable	Levels of F^- concentration in irrigation water (mg L^{-1})					
		0	50	150	400	800	Mean
Bean	Beans (g of DM $plant^{-1}$)	3.9 bc	10.8 a	10.3 ab	9.8 ab	1.9 c	5.3
	Pods (g of DM $plant^{-1}$)	3.3 a	7.3 a	6.3 a	4.8 a	2.1 a	4.8
	Number of pods (n $plant^{-1}$)	16 b	46 a	32 ab	20 b	17 b	26
	Pod length (cm)	7.8 a	7.9 a	7.9 a	8.1 a	6.4 a	7.6
	Leaves (g of DM $plant^{-1}$)	8.8 b	15.2 a	13.8 a	12.7 ab	8.8 b	11.9
	Stem (g of DM $plant^{-1}$)	5.2 b	10.7 a	9.5 a	7.9 ab	5.9 b	7.8
	Plant height (cm)	41 a	55 a	52 a	60 a	49 a	51
	Number of leaves (n $plant^{-1}$)	17 c	37 a	34 ab	35 ab	22 bc	29

Means followed by the same letter are not significant different for P≤0.05.

Soil water-soluble fluoride

The initial soil water-soluble fluoride content was 8.5 ± 2.2 mg kg^{-1} which was significantly lower than the limit of 16.4 mg kg^{-1} of available fluoride established by EPA, FAO, and WHO as cited by Lakshmi et al., (2017a), Limón-Pacheco et al. (2018), and Paul et al. (2011). At the contrary, water-soluble fluoride found in the soil at the end of the experiments was found to be always significantly higher than the mentioned limit.

Positive significant correlations were observed between the total amounts of fluoride supplied by contaminated irrigation waters per kg of soil (Table 18) and the water-soluble fluoride of the soil in which maize ($R^2 = 0.74$), tomato ($R^2 = 0.80$) and bean ($R^2 = 0.93$) were grown (Figure 5).

Also the one-way ANOVA data analyses showed that, for all the investigated crops, the soil water-soluble fluoride significantly increase with increasing fluoride concentration in treated water with the sharpest trend observed for bean ($P<0.05$) (Table 19).

Table 18 - Quantity of F^- supplied by irrigation water at different levels of F^- concentration (0, 50, 150, 400, 800, 1600 mg $F^- L^{-1}$) per kg of substrate for the three investigated crops (maize, tomato and bean).

Levels of F^- concentration in irrigation water (mg L^{-1})	**mg of F^- supplied by irrigation water kg^{-1} of substrate**		
	Maize	**Tomato**	**Bean**
0	0.0	0.0	0.0
50	15. 5	35.0	32.4
150	46.5	104.9	97.2
400	124.0	279.7	259.3
800	248.1	559.3	518.6
1600	496.2	-	-

Data for tomato and bean at the level 1600 mg $F^- L^{-1}$ are not reported since no plants survived for this treatment.

Table 19 - Soil water-soluble fluoride (mg F^- kg^{-1}) at the end of the experiments in relation to the different levels of F^- concentration (0, 50, 150, 400, 800, 1600 mg F^- L^{-1}) supplied with irrigation water for the three investigated crops (maize, tomato and bean).

Levels of F^- concentration in irrigation water (mg L^{-1})	Soil water-soluble fluoride mg F^- kg^{-1}		
	Maize	Tomato	Bean
0	9.7 d	6.0 d	9.8 e
50	23.8 d	28.3 cd	43.4 d
150	62.1 cd	67.8 bc	99.0 c
400	101.3 bc	107.1 ab	125.6 b
800	139.1 ab	146.6 a	225.1 a
1600	176.3 a	-	-

Means followed by the same letter are not significant different for P≤0.05.

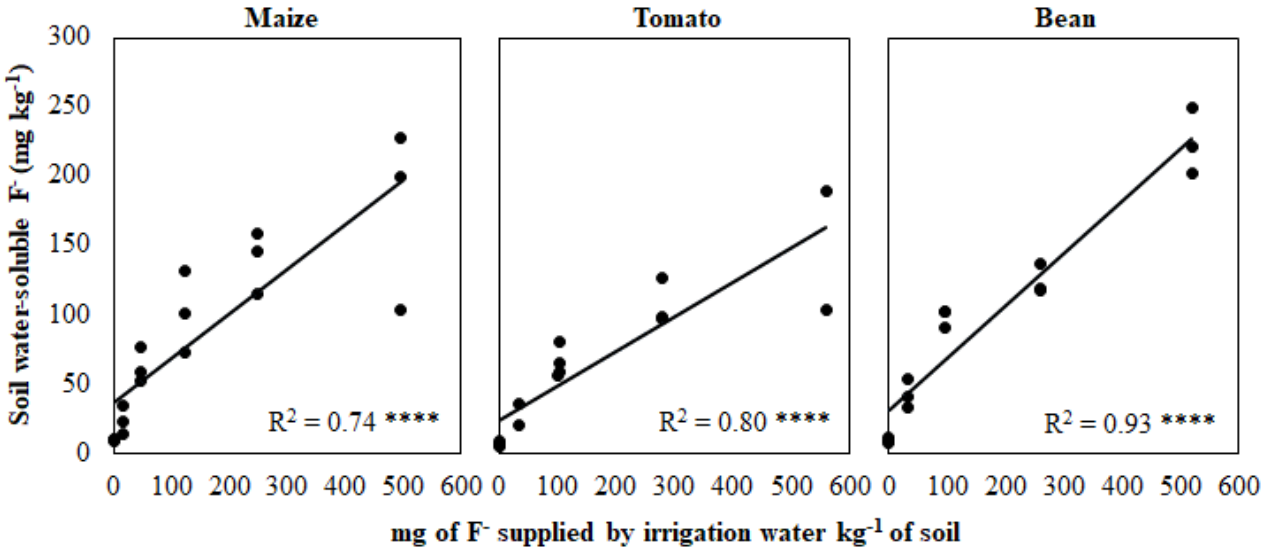

Figure 5 - Relationship between water-soluble F^- concentrations in soil (mg kg^{-1}) and the total amounts of F supplied by irrigation water at different levels of F^- concentration (0, 50, 150, 400, 800 and 1600 mg F^- L^{-1}) per kg of soil (mg kg^{-1}) for the three investigated crops (maize, tomato and bean). **** P<0.0001; ***: P<0.001; **: P<0.01; *: P<0.05; n.s.: not significant.

Fluoride uptake and partitioning

Fluoride concentrations in different plant parts (leaves, stems, pods and grains/fruits) of maize, tomato and bean are reported in Table 20. A significant interaction treatment×plant part was observed in the accumulation of F^- in all studied crops.

For all three species, the F^- concentration of edible parts (grains, fruits or beans) and also pods for bean, was not affected by the treatments. Regarding crop residues (stem and leaves), at the contrary, increasing F^- concentrations were detected for rising levels of F^- in irrigation water. However, significantly higher F^- accumulations with respect to the control were observed mainly for plants treated with the F^- levels.

In the case of maize, the F^- concentration in crop residues of plants irrigated with 800 and 1600 mg F^- L^{-1} was about 8 and 11 times higher than that of control plants residues, respectively. Leaves of plants treated with 150, 400 and 800 mg F^- L^{-1} accumulated around 12, 25 and 38 times more F^- than control plants, in the case tomato, and about 2.6, 8 and 15 times in the case of bean. Tomato and bean stems showed F^- concentrations 4 and 5 times (for tomato) and 4 and 7 times (for bean) higher than the stems of the control plants when plants were irrigated with 400 and 800 mg F^- L^{-1}.

Since F^- accumulation of stem and leaves was rising with the treatments, whereas that of edible parts seemed unaffected, the accumulation pattern between plant organs was different among the plants treated with increasing levels of F^-. In the case of maize no significant differences were observed between F- concentration in grains and crop residues (stem and leaves) of plants irrigated with 0, 50 and 150 mg F^- L^{-1}, while leaves and stem showed a F^- accumulation from 2 to 5 times higher than grains when the level of F^- in irrigation water increased at 400, 800 and 1600 mg F^- L^{-1}. A prevalent accumulation of F^- in leaves compared to other organs was observed in bean and tomato for F^- levels in irrigation water from 150 mg F^- L^{-1} onwards. In bean, stems and pods of plants irrigated with 1600 mg F^- L^{-1} showed a F^- accumulation 2-3 times greater than seeds.

Table 20 - Total F concentration in various plant parts samples (leaves, stem, pods and grains/fruits) of maize, tomato and bean collected at the maturity stage (mg kg^{-1} of dry matter) from plants irrigated with water at different levels of F^- concentration (0, 50, 150, 400, 800, 1600 mg F^- L^{-1}).

Crop	Plant part	Levels of F^- concentration in irrigation water (mg L^{-1})					
		0	50	150	400	800	1600
Maize	Stem and leaves	4.2 Ca	8.7 Ca	13.9 Ca	18.4 BCa	34.9 ABa	46.9 Aa
	Grains	9.4 Aa	12.6 Aa	12.4 Aa	10.6Ab	7.9 Ab	9.6 Ab
Tomato	Leaves	3.5 Da	10.4 Da	40.8 Ca	87.0 Ba	131.5 Aa	n.a.
	Stem	4.7 Ba	4.4 Ba	6.2 Bb	19.0 ABb	24.4 Ab	n.a.
	Fruits	15.4 Aa	13.4 Aa	12.2 Ab	8.0 Ab	11.2 Ab	n.a.
Bean	Leaves	12.1 Da	22.0 CDa	31.6 Ca	94.3 Ba	181.8 Aa	n.a.
	Stem	3.2 Ba	9.7 ABab	8.0 Bb	12.4 ABb	23.5 Ab	n.a.
	Pods	12.9 Aa	12.2 Aab	13.4 Ab	14.7 Ab	17.7 Abc	n.a.
	Seeds	8.4 Aa	7.1 Ab	9.2 Ab	9.5 Ab	8.6 Ac	n.a.

Means followed by the same letter were significant different for $P \leq 0.05$; capital letters refer to comparisons among levels of F^- in irrigation water within plant parts, lower-case letters to plant parts within levels of F^-.

Positive significant correlations between soil water-soluble fluoride and total F^- in plant tissues were found for leaves and stems of the three crops and for pods in bean. Concerning edible parts, at the contrary, since the accumulation of F^- in their tissue did not increase with increasing soil water-soluble fluoride, correlations between the two variables were not significant, while in the case of tomato a negative correlation was found (Figure 6).

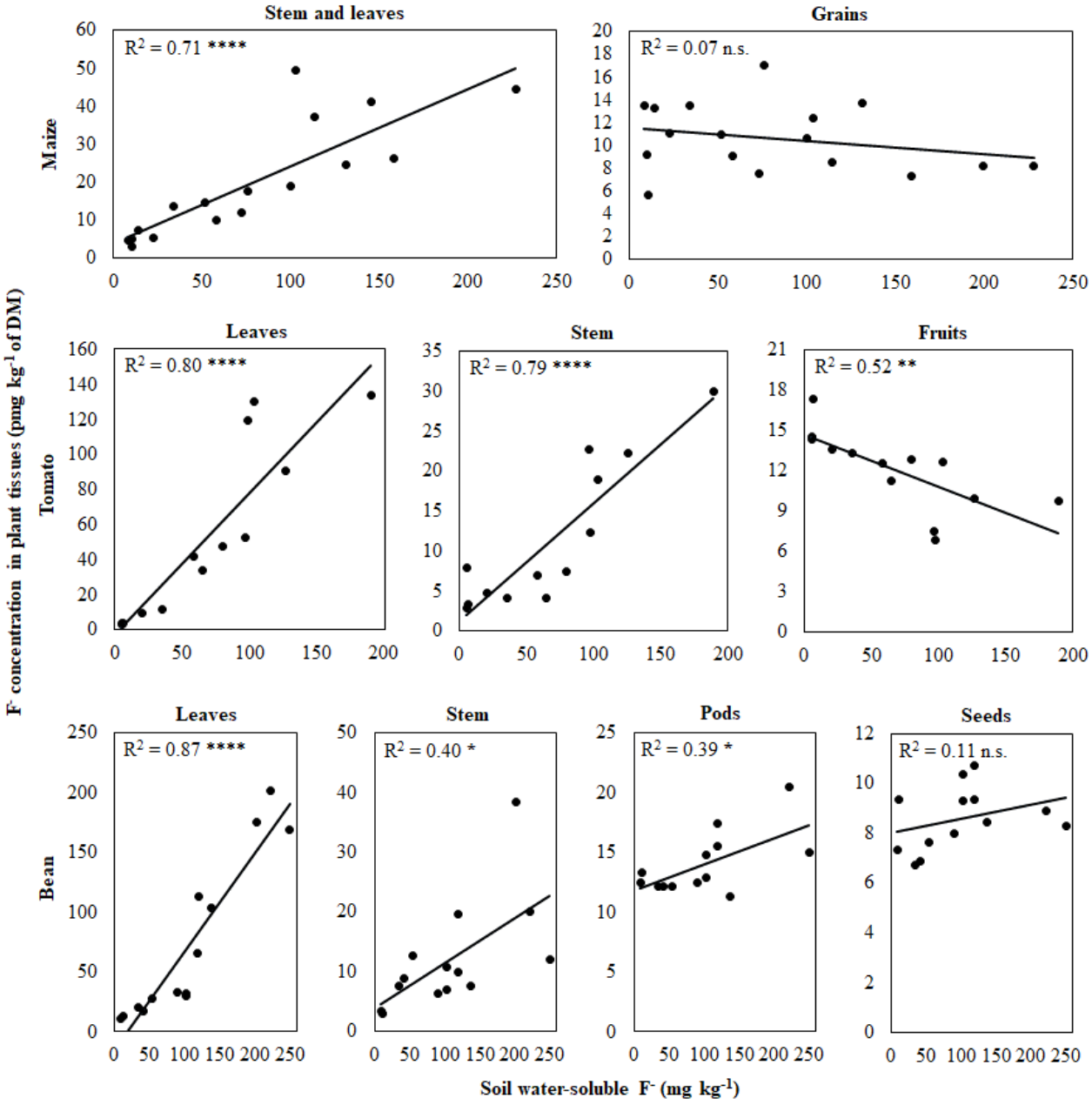

Figure 6 - Relationship between water-soluble F^- concentrations in soil (mg kg^{-1}) and the total F^- concentration in various plant parts (leaves, stems, pods and grains/fruits) of maize, tomato and bean collected at the maturity stage (mg kg^{-1} of dry matter) from plants irrigated with water at different levels of F^- concentration (0, 50, 150, 400, 800, 1600 mg F^- L^{-1}). **** P<0.0001; ***: P<0.001; **: P<0.01; *: P<0.05; n.s.: not significant.

Bioconcentration factors (BCF)

Bioconcentration factors were calculated for all plant parts of the studied crops in order to evaluate the fluoride accumulation in each organ with respect to the soil water-soluble fluoride. Ratios between BCFs of the different plant parts are also reported in Table 21.

In the crop residues of maize (stem and leaves), BCF values varied from 0.18 to 0.44 without significant differences among treatments. BCFs of grains showed a decreasing trend with rising concentration of F^- in the irrigation water ranging from 1.01 to 0.07. As can be observed from the BCF ratios between the two parts, grains of plants irrigated with 0 and 50 mg F^- L^{-1} showed BCF values 2.3 and 1.6 times greater than those of crop residues, respectively. At the opposite, for plants irrigated with 800 and 1600 mg F^- L^{-1} the BCF ratios were shifted in favour of greater bioconcentration in crop residues compared to grains. BCF values for tomato leaves, stems and fruits varied in the ranges 0.59-0.98, 0.09-0.82 and 0.09-2.56, respectively. As seen for maize, also in the case of tomato, the BCF ratios were in favour of fruits for plants irrigated with 0 and 50 mg F^- L^{-1} and gradually moved towards greater values in leaves with increasing concentration of F^- in irrigation water. BCFs of leaves were found to be around 5 and 10 times higher than those of stem and fruits for plants irrigated with 800 mg F^- L^{-1}, respectively.

Concerning bean, with the exception of the control treatment, leaves were always the organs with the highest BCFs, concentrating the fluoride from 2.5 to 10 times more than in the stems, from 1.7 to 10 times more than in the pods and from 3.3 from 20 times more than in the beans. The imbalance in fluoride accumulation in favour of leaves with respect to the other organs increased with the increasing availability of fluoride for plants. Only in the case of control treatment pods showed a BFC not significantly different from that of leaves.

Table 21 - Bioconcentration factors for different plant parts (leaves, stems, pods and grains/fruits) of maize, tomato and bean irrigated with water at different levels of F^- concentration (0, 50, 150, 400, 800 and 1600 mg F^- L^{-1}).

Crop	BCF	Levels of F^- concentration in irrigation water (mg L^{-1})					
		0	50	150	400	800	1600
Maize	BCF Stem and leaves (S+L)	0.44 Ab	0.38 Aa	0.23 Aa	0.18 Aa	0.26 Aa	0.34 Aa
	BCF Grains (G)	1.01 Aa	0.61 Ba	0.20 Ca	0.10 Ca	0.06 Ca	0.07 Ca
	BCF ratio S+L : G	1 : 2.3	1 : 1.6	1 : 0.9	1 : 0.6	1 : 0.2	1 : 0.2
Tomato	BCF Leaves (L)	0.59 Bb	0.89 ABa	0.61 ABa	0.82 ABa	0.98 Aa	n.a.
	BCF Stem (S)	0.82 Ab	0.35 Bb	0.09 Bb	0.18 Bb	0.17 Bb	n.a.
	BCF Fruits (F)	2.56 Aa	1.10 Ba	0.18 Cb	0.07 Cb	0.09 Cb	n.a.
	BCF ratio L : S : F	1 : 1.4 : 4.3	1 : 0.4 : 1.2	1 : 0.2 : 0.3	1 : 0.2 : 0.1	1 : 0.2 : 0.1	
Bean	BCF Leaves (L)	1.17 Ab	0.51 Ca	0.32 Da	0.75 Ba	0.82 Ba	n.a.
	BCF Stem (S)	0.31 Ac	0.22 Ab	0.08 Bb	0.10 Bb	0.11 Bb	n.a.
	BCF Pods (P)	1.25 Aa	0.29 Bb	0.13 Cb	0.12 Cb	0.08 Cb	n.a.
	BCF Beans (B)	0.80 Ab	0.17 Bb	0.09 Bb	0.08 Bb	0.04 Bb	n.a.
	BCF ratio L : S : P : B	1: 0.3: 1.1: 0.7	1: 0.4: 0.6: 0.3	1: 0.2: 0.4: 0.3	1: 0.1: 0.2: 0.1	1: 0.1: 0.1: 0.05	

Means followed by the same letter are not significant different for $P \leq 0.05$; capital letters refer to comparisons among levels of F^- in irrigation water within plant parts, lower-case letters to plant parts within levels of F^-.

Discussion

The contamination source is a relevant aspect to consider in the assessment of fluoride effects on crop fluoride accumulation and crop yield. There are two main pathways through which fluoride can enter into plants. Gaseous fluoride can be uptaken by leaves through stomatal diffusion, whereas fluoride in the soil solution is absorbed from roots by passive diffusion processes and transported via xylem into shoots (Baunthiyal and Ranghar, 2014, Singh, et al., 2018, Yadu, et al., 2016). Fluoride ions circulating in the soil solution can derive directly from the solubilisation process of rich-fluorine soil minerals or they can be supplied by irrigation water. The impacts of the soil contamination on crops could be different to some extent from those deriving from the use of polluted water. Despite this,

given the lack of studies specifically focused on the effects of contaminated irrigation waters on the crops of interest, works regarding direct soil pollution were anyway considered in the present study.

Effects of fluoride on crop yields

Szostek and Ciecko (2017b) noted that maize aerial biomass progressively increased when the soil was contaminated with 0, 100, 200 and 300 mg F^- kg^{-1} of soil. This trend was partially confirmed by the results observed in the present study. An increase of the aerial biomass was in fact detected for increasing F^- concentration in irrigation water, reaching the highest value for plant irrigated with 400 and 800 mg F^- L^{-1} while plants treated with 1600 mg F^- L^{-1} did not show any difference compared to control plants. Concerning grain yields, values found by Cui et al. (2011) ranged between 42.4 and 6.2 (g $plant^{-1}$), with a reduction from 9.9% to 85.4% with respect to control, when doses of soil fluoride ranged from 100 to 1500 mg F^- kg^{-1}. At the opposite, values observed in the present work, ranging from 24.5-47.4 (g $plant^{-1}$), were not significantly affected by treatments. Such differences may be attributed both to a higher degree of tolerance acquired by the local maize variety utilized in the present study and also to the fluoride distribution modality. In the case of direct soil contamination, in fact, the soil has already the highest fluoride concentration at the stage of the cob development, rather than a progressive fluoride accumulation obtained with the contaminated irrigation.

Regarding tomato, no literature was found on the effects of fluoride in soil solution on growth and yield of adult plants. However, studies conducted on tomato seedlings found a negative impact of fluoride contaminated nutrient solutions or soil on the growth and development of plants in the very early stages, with different degrees of tolerance among varieties (Ahmad, et al., 2018, Bar-Yosefk, 1988, Stevens, et al., 1998). This appear to be consistent with the significant reduction in the crop residue biomass, up to 68%, observed in this study for the increasing concentration of fluoride in

irrigation water. In spite of that, the present study highlights that treatments did not affect the tomato fruits production.

In the case of bean, although some literature was found on the effects of atmospheric fluoride on its growth, no studies exist regarding fluoride toxicity when the contamination concerns the soil or the nutrient solution. In a study on mung bean seedlings (*Vigna radiata* (L.) R. Wilczek), Yu (1996) found a significant increase in the weight of cotyledons for F concentrations ranging from 0 to 95 mg F^- L^{-1} while the radicles weight was reduced. In contrast, Sabal et al. (2006) observed significant decreases in seed germination %, total biomass and shoot and root length of cluster bean seedlings (*Cyamopsis Tetragonoloba* (L.) taub.) when exposed at increasing F concentration. Consistently, Bustingorri and Lavado (2014) detected a 13% and 40% reduction of biomass compared with control, when soybean plants (*Glycine max* (L.) Merr.) were grown in 200 or 450 mg kg^{-1} total soil F, respectively.

The growth and biomass stimulation observed in this study both in maize and bean plants irrigated with water respectively up to 400 and 800 mg F^- L^{-1} may be due to a fluoride induced gibberellin-mimic action or to the formation of new gibberellins promoted by this ion as supposed by Ballantyne (1989). This mechanism may also represent an explanation of the, even if not significant, increasing calibre of tomato fruits with increasing fluoride exposure doses. According to Crozier and Turnbull (1984) gibberellin may stimulate cellular distention by altering the distribution of calcium ions that move from the plant cell wall to the protoplast. Garrec and Chopin (1982) supposed that, similarly, fluoride may induce a migration of calcium ions towards sites where fluoride accumulates and this could lead to an increase in cell wall plasticity and elongation of plant tissues.

On the other hand, the depression of biomass production observed in the crop residues of tomato plants with application of increasing fluoride doses, may be due to the prevalence of fluoride toxic effects with respect to its stimulating action. Fluoride has been shown, in fact, to produce detrimental

effects on plant metabolism, inhibiting photosynthesis, inducing oxidative stress and interfering with enzymatic activities, protein synthesis and gene expression patterns also without the formation of any visible symptom of damage on plant tissues (Choudhary, et al., 2019). The mechanisms that regulate the tolerance/sensitivity to fluoride of different plants species and varieties are not yet fully understood. A higher degree of tolerance has been hypothesized to be related with a greater ability of some plants to deactivate F as for example by shifting to F-insensitive metabolic pathways. Reactions with organic compounds may remove fluoride from sites of enzyme inhibition; fluoride may be also inactivated by means of reaction with cationic sites, or it can be sequestrated into vacuoles or translocated to the exterior leaf surface (Baunthiyal and Ranghar, 2015).

Effect of fluoride in the irrigation water on soil water-soluble fluoride

The significant rise of the soil water-soluble fluoride after the regular irrigation with contaminated waters, as it was observed in this study, confirms previous results obtained by other authors (Chakrabarti, et al., 2013b, Jha, et al., 2013). All the values found in soils after the treatments were always significantly higher than the limit of 16.4 mg kg^{-1} of soil available fluoride recommended by EPA, FAO, and WHO as cited by Lakshmi et al., (2017a), Limón-Pacheco et al. (2018), Paul et al. (2011) ($P<0.05$). Therefore, these findings highlight that the extended use of high-fluoride contaminated waters can significantly affect the quality of agricultural soils.

Fluoride concentration in plant tissues, accumulation patterns and bioconcentration factor (BCF)

For all three studied species, it was observed that an increase in the concentration of fluoride in the irrigation water corresponds to a decrease in the BCFs of edible parts (fruits/grains) with a tendency of the plant to accumulate a higher amount of fluoride in leaves (or leaves and stem for maize) with

respect to other aerial organs. This trend may be interpreted as an attempt of the plant to protect reproductive organs and embryos from excessive fluoride toxicity.

Concerning maize, Szostek and Ciecko (2014) observed increasing fluoride concentration, ranging from 1.8 to 19.4 mg F^- kg^{-1} DM, in the total aerial biomass of plants cultivated in soils contaminated with rising levels of fluoride (0, 100, 200 and 300 mg F^- kg^{-1}). A growing trend was also reported by Cui et al. (2011) in leaves, stem and grains of maize plants exposed to six levels of soil contamination (0, 100, 200, 500, 1000 and 1500 mg F^- kg^{-1}). This tendency was only partially confirmed by our results, since rising concentrations of fluoride were detected in the case of stem and leaves but not in grains, that instead, did not show significant differences in the accumulation between the fluoride exposure doses.

The accumulation pattern among aerial organs reported by Cui et al. (2011) was the same for all the six levels of soil contamination (0, 100, 200, 500, 1000 and 1500 mg F^- kg^{-1}): leaves > stem > grains; with leaves accumulating from 9 to 11 times more fluoride than the stem and from 46 to 77 times more than the grains. A greater concentration in crop residues (stem and leaves) with respect to grains (4-5 times higher) was confirmed in our study just when plants were irrigated with 800 and 1600 mg F^- L^{-1} while, for lower fluoride doses, grains and crop residues accumulated at the same extent.

In order to evaluate results from different studies, a comparison among the BCFs rather than among the pure values of concentration in tissues would be preferable. The assessment of the fluoride accumulation, in fact, should consider this parameter with respect to the fraction of fluoride available in the soil for plants. Since data on soil water-soluble fluoride were not reported by the abovementioned studies the calculation of BCFs was not possible. Taking the above into account, it is still worth to consider that, ranging between 0.9 and 1.7 mg F^- kg^{-1} DM, values of fluoride accumulation in grains found by Cui et al. (2011) were from 5 to 13 times lower than those observed in our study.

Regarding tomato, in a large study conducted in West Bengal, India, by Bhattacharya et al. (2017), fruits of plants grown in the districts of Bankura and Purulia showed values of fluoride concentration in their tissues of 7.1 and 8.5 mg F^- kg^{-1} DM, respectively. The soil total fluorine of the two areas was on average around 132 and 182 mg F^- kg^{-1}. Values of soil water-soluble fluoride were not reported, therefore the BCFs were not possible to determine. Also in West Bengal, in the village of Junitpur, Birbhum district, Gupta and Banerjee (2011) found a fluoride concentration in tomato fruits of 8.8 mg F^- kg^{-1} DM. The average soil water-soluble fluoride was 2.6 mg F^- kg^{-1} and levels of fluoride in the irrigation water ranged from 0.6 to 4.1 mg F^- L^{-1}. The BCF value deriving from these data was 3.3. Fluoride accumulation reported by the these authors are consistent with the range of values found in our study for tomato fruits (8.0-15.4 mg F^- kg^{-1} DM) and with the BCF value of 2.6 found in the control treatment, suggesting a tendency of tomato plants to hyper-accumulate fluoride in fruits (BCF > 1) when low values of water-soluble fluoride are detected in the soil.

Lower contents of fluoride (1.4 and 2.3 mg F^- kg^{-1} DM) were found by Lakshmi et al. (2017a) in the fruits of tomato grown in two villages of Lingotam and Bendalpahad in the area of Narkatpally Mandal (Nalgonda district, Telangana, India). Also in this case, it was impossible to calculate the BCFs but total fluoride concentrations in the soil (3.0 and 4.7 mg F^- kg^{-1}) and in the water (1.4 and 2.5 mg F^- L^{-1}) were generally very low.

Concerning bean, in a study involving nine agricultural fields in Nagarparkar district, Pakistan, Brahman et al. (2014) found that values of fluoride concentration in kidney bean seeds ranged from 26.3 to 36.9 mg F^- kg^{-1} DM. Levels of total and water-soluble fluoride in soil were between 125-566 and 3.9-18.6 mg F^- kg^{-1}, respectively, while the total fluoride in water varied from 18.5 to 35.5 mg F^- L^{-1}. The BCF values, calculated on the basis of soil water-soluble fluoride ranged from 2 to 8.2. The concentration of fluoride in bean seeds observed by Gupta and Banerjee (2011) in Junitpur, was 15.3 mg F^- kg^{-1} DM with a BCF of 5.8. The apparent hyper-accumulating behaviour that emerges from

those two studies was not confirmed in our case, with fluoride in bean seeds ranging from 8.4 to 9.5 mg F^- kg^{-1} DM and BCFs varying from 0.04 to 0.80.

In general for the three studied crops, all the differences observed among studies may be ascribed to differences in the fluoride dose and distribution modality, the peculiar uptake capacity of each variety as well as the soil features and other growth conditions.

Conclusions

Among the investigated crops, the greater tolerance to fluoride was observed in maize and bean. The total aerial biomass of these two crops, in fact, was not affected when compared to the control treatment by any level of fluoride in irrigation water, being instead stimulated by rising fluoride doses, up to 800 mg F^- L^{-1} in the case of maize and up to 400 mg F^- L^{-1} in bean. In tomato, although the crop residue biomass (stem and leaves) was reduced by increasing fluoride levels, the production of fruits was not affected. Fluoride concentration in edible parts ranged between 7.9-12.4, 8.0-15.4 and 7.1-9.5 mg F^- kg^{-1} DM for maize, tomato and bean, respectively, without any significant difference between treatments. Nevertheless, the present study highlighted that the protracted use of high-fluoride contaminated waters can considerably affect the quality of agricultural soils leading to a significant rise of the soil water-soluble fluoride content. Furthermore, for all the studied species, fluoride accumulation in crop residues significantly increased with rising concentration of fluoride in the irrigation water, up to 11 times in maize, 37 times in tomato leaves and 15 times in bean leaves with respect to the control treatment, representing an indirect risk for human health. In fact, in the contaminated areas of the East African Rift Valley, harvested crop residues of maize and bean are mainly used for feeding livestock so that fluoride may enter the food chain through the consumption of animal products. When the residues are left in the field or mulched, as in the case of tomato, they

can contribute to increase the level of fluoride of the top soil, likely exacerbating the fluoride contamination.

For all the above, crops whose edible organs are leaves (e.g. kales, spinach, lettuce etc.) are also worth to be investigated. Moreover, further experiments are needed to assess the interaction between soil and water contamination in the bioaccumulation of fluoride in food crops and to test whether the consumption of products (e.g. milk and meat) from animals fed with fluoride-rich crop residues may represent an actual risk for human health. Finally, and not least, possible mitigation strategies (e.g. the use of soil amendments, phytoremediation etc.) need to be identified and tested to reduce the impact of fluoride on food and feed production.

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Chapter IV

General conclusions

General conclusions

The analysis of literature highlighted that the cultivation of food crops in areas affected by soil and water fluoride contamination can represent a potential risk to human health. Several studies, in fact, pointed out that, in addition to the consumption of fluoride-rich waters, the intake of contaminated foods can significantly contribute to increase the daily fluoride ingestion of individuals, enhancing the risk of overcoming the recommended dose and incurring fluorosis diseases. Although the assessment of crop fluoride accumulation has been addressed by several authors, mostly in Asia, the literature review brought to the light that in some other seriously affected regions, such as for example the East African Rift Valley, the fluoride contamination of food crops is still understudied. The analysis of the studies conducted in controlled conditions revealed that, in general, plants tend to accumulate fluoride mostly in the root system, to the disadvantage of those crops whose edible part is the underground organs, as for example radish. This tendency was confirmed, in the majority of cases, by the results of our field experiments, except in the case of tomato in which the accumulation of fluoride in roots and leaves was not significantly different.

However, when plants are exposed to increasing doses of fluoride, the accumulation in other edible parts (e.g. leaves, fruits or seeds) was observed to reach considerable levels as also observed in the field and pot trials that we conducted.

The high water-soluble fluoride content observed in agricultural soils of rural areas of North Tanzania was reflected in a substantial accumulation of fluoride in plants edible parts. Average concentrations of 14.2, 11.4, 11.3 and 8.0 ppm were in facts observed for kale, tomato, bean and maize respectively, demonstrating that the considered food items, that are among the most consumed in the rural area under study, substantially contribute to fluoride-correlated diseases, especially in earlier ages.

The protracted use of high-fluoride contaminated waters along the crop cycle, as tested in the greenhouse pot experiment, was observed to considerably affect the quality of the soil leading to a

significant rise of its water-soluble fluoride concentration. Moreover, for all the studied species, the fluoride accumulation in crop residues increased with increasing fluoride concentration in the irrigation water. This may represent an indirect risk for human health, since in the rural areas of the East African Rift Valley crops residues of maize and bean are mainly used for feeding livestock from which fluoride could enter the food chain. When residues are left in the field or mulched, as in the case of tomato, instead, this can contribute to increase the fluoride content of the top soil, exacerbating the fluoride contamination.

For all above, future perspectives for further investigations may include: (i) to test the impacts of fluoride on crops whose edible organs are roots, tubers and bulbs (e.g. carrots, potatoes, onion and turnip) or leaves (e.g. kales, spinach, lettuce etc.), (ii) to the assess the interaction between soil and water contamination in the bioaccumulation of fluoride in food crops, (iii) to test whether the consumption of products (e.g. milk and meat) from animals fed with fluoride-rich crop residues may represent a substantial risk for human health, (iv) to identify and test possible mitigation strategies (e.g. use of soil amendments, phytoremediation etc.) to reduce the impact of fluoride on food and feed production.