Journal of the
Science of
Food and Agriculture

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

Journal:	*Journal of the Science of Food and Agriculture*
Manuscript ID	JSFA-19-3580.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	04-May-2020
Complete List of Authors:	Rizzu, Margherita; University of Sassari Department of Agriculture, Department of Agriculture and Desertification Research Centre Tanda, Alberto; University of Sassari Department of Agriculture, Department of Agriculture and Desertification Research Centre Canu, Linda; University of Sassari Department of Agriculture, Department of Agriculture and Desertification Research Centre Masawe, Khairia; Nelson Mandela African Institution of Science and Technology, Lifes Sciences and Bioengineering Mtei, Kelvin; Nelson Mandela African Institution of Science and Technology, Lifes Sciences and Bioengineering Deroma, Mario; University of Sassari Department of Agriculture, Department of Agriculture Roggero, Pier ; University of Sassari Department of Agriculture, Department of Agriculture and Desertification Research Centre Seddaiu, Giovanna; University of Sassari Department of Agriculture, Department of Agriculture and Desertification Research Centre
Key Words:	Maize, Tomato, Bean, Kale, Soil contamination, Translocation Factor

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

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

Margherita Rizzu[a,b,*,†], Alberto Tanda[a,b,†], Linda Canu[a], Khairia Masawe[c], Kelvin Mtei[c], Mario Antonello Deroma[a], Pier Paolo Roggero[a,b], Giovanna Seddaiu[a,b]

[a] Dipartimento di Agraria, University of Sassari, Viale Italia 39, 07100, Sassari, Italy

[b] Desertification Research Centre, NRD, University of Sassari, Viale Italia 39, 07100, Sassari, Italy

[c] Nelson Mandela African ~~Institute~~ Institution of Science and Technology, NM-AIST, Arusha, Tanzania

† Margherita Rizzu and Alberto Tanda should be considered joint first author

*Corresponding author: Margherita Rizzu

Tel.: +39 3454034148

E-mail: mrizzu@uniss.it

Address: Viale Italia 39, 07100, Sassari, Italy;

Abstract

BACKGROUND: 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.

RESULTS: Average fluoride contents of 8.0, 11.4, 11.3 and 14.2 ~~ppm~~ mg kg^{-1} of dry matter were observed respectively for maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris* L.) and kale (*Brassica sp. pl.*) edible parts. The cumulative estimated average daily dose (EADD) ranged from 0.026 to 0.165 mg F d^{-1} kg^{-1} among different rural 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.

CONCLUSIONS: 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, Translocation Factor

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 [1]. Particularly concerning fluorine (F), Kloos and Haimanot [2] 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 [3], such as 100 mg L^{-1} or more measured in Tanzania by Ghiglieri ~~et al~~*et al*. [4]. 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 [4]. 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 [5]. Excessive and continuous intake of this element in the human diet can lead, in fact, to a series of severe diseases known as fluorosis [6]. The fluoride introduced with the diet is assimilated into teeth and bones [7] forcing out hydroxide ions from hydroxyapatite crystal with the formation of a tougher and stronger new mineral: fluorapatite [8]. 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 [1]. 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~~ low IQ [9, 10].

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~~Argentine, fluoride contamination in groundwater is a crucial matter [11]. In EARV about 90% of the population had shown fluorosis symptoms [12] and in the region of Arusha in Tanzania the prevalence of dental fluorosis is about 100% [13]. 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 [3].

Most health studies regard chronic fluoride exposure from drinking water. However, food can also be responsible for another potentially significant pathway [5]. 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 however available in the 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 [14].

The main natural source of fluoride in the agricultural soil is represented by the disruption of fluoride-rich minerals from the bedrock [15]. 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 [16]. 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 and exchangeable fluoride content which is considered ~~is~~ biologically active for plants and animals while other fractions have been reported to have low or no bio-availability [17]. Plants roots passively absorb the ~~soluble~~ available fluoride through the soil water solution and then it is moved via xylematic conducts up to the stems, leaves and fruits [18]. 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 [19], but many studies highlighted a higher concentration of fluoride in roots than the other plant parts [20-23].

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) [24]. 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 1. Soil textures vary from loamy sand to sandy loam. Hydrological parameters were derived following Saxton [25] 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 [26].

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~~ is characterized by a higher variability of rain intensity and distribution and it is named "Vuli" or short rainy season (210 mm~~/~~ y^{-1}). In the rest of the year, dry periods prevail [27]. 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 [28]. The survey highlighted that most of the agricultural land in the three sites was cultivated with maize (*Zea mays* L.) as the 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 [29], 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.

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). Plot sizes for each crop are reported in Table 2.

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 the middle of 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~~(Table 3).

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 h~~ours~~ at 60ºC and powdered with a Retsch ZM 200 (Retsch, Haan, Germany).

Chemical analyses

For total F determination in plant tissues, powdered vegetable samples (0.2 g) were digested with 2 ~~ml~~ mL of nitric acid ~~67%~~(15 mol L^{-1}), 3 mL of hydrogen peroxide ~~at 30%~~ (~~3~~ 10 mol L^{-1})~~ml)~~ and 5 mL of 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 (8 mol L^{-1}),~~M)~~ 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~~Water- ~~soluble~~ Soluble ~~fluoride~~F (WS-F) extraction, 2 g of sieved soil were shaken in 40 ~~ml~~ mL of deionized water (ratio 1:20) for 16~~-~~–18 h. The extraction fluid/sample mixture was centrifuged and a 10 ~~ml~~ mL aliquot from the supernatant was removed for the estimation of F.

Different forms of F in soil (Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and residual F (Res-F)) were determined by sequential extraction following the method by Berger *et al.*[30]. Prior to the Ex-F extraction, the hot water-soluble F fraction was removed (by shaking 0.5 h at 60°C; soil:solution ratio = 1:10) in order to discriminate the Ex-F from the soluble fraction. Res-F was determined on the residue with the method advised by Geretharan *et al.*[31]. The Total F was obtained by summing the different F fractions.

Both F concentration of the plant samples digest solution and soil samples ~~water~~ extraction solutions were determined potentiometrically using a fluoride ion-selective electrode (ISE - Thermo Scientific Orion 9609BNWP; measurement range: from 10^{-6} mol L^{-1} to a saturated F^- solution). 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~~ 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 [32] :

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

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

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 [33]:

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

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 [34] reports that fluoride is absorbed in a range between 75 % and 100 % during the digestion process, these two limits w~~h~~ere both assessed in two different scenarios.

The IR was estimated as follow:

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

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 [28] 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 [35]

where RfD is the reference dose for humans associated with the 'no adverse effect level' (NOAEL). USEPA [36] recommended a value of RfD for fluoride equals to~~, 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 [37] 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} [23, 38, 39] 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. A factorial ANOVA was also performed to analyse the data concerning soil fluoride fractions, where locations and the different F-fractions were considered as random factors. Multiple comparisons of means ~~related to fluoride partitioning in plant tissues~~ were then performed using a ~~Tukey test~~Fisher's LSD test. The ANOVAs ~~was~~ were performed with the R software [40]. The correlation between each soil fluoride fraction and the concentration of fluoride in plants tissues was analysed using Pearson's correlation coefficient test.

Results

Distribution of ~~Soil water-soluble~~ fluoride fractions in the soil

Average concentrations of ~~S~~soil ~~water-soluble fluoride~~WS-F in the 0-40 cm layer ~~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 [23, 38, 39] (Table ~~3~~4).

The concentrations of WS-F, Ex-F, Fe/Mn-F, OM-F and Res-F in the topsoil (0-20 cm) are illustrated in Figure 1. The fraction distribution coefficients (FDCs) are obtained by the ratio of each F fraction and the TF [41] (Table 5). A significant site x fluoride fraction interaction was observed in terms of both absolute values and FDCs. The soil F contamination of all the three study sites was mainly associated with the Res-F, as indicated by the highest values of FDCs (from 69.4% to 94.0%), and with the WS-F (form 4.9% to 20.3%). The OM-F was the third most represented fraction, together with the Ex-F in Momela and the Ex-F and Fe/Mn-F in Olkung'wado. In all the three sites Ex-F and Fe/Mn-F represented a very marginal percentage (<0.8 %) of the overall fluoride. In Uwiro, in spite of the lowest value of TF (551.7 mg kg^{-1}), the highest WS-F and OM-F, and the lowest Res-F, were observed in terms of both absolute values and percentages over the total (FDCs). On the contrary, Olkung'wado, which was the site with the highest TF (689.1 mg kg^{-1}), was characterized by the lowest WS-F in terms of FDCs (which was, however, not significantly different from the WS-F in Momela in absolute terms) and the highest Res-F in absolute and percentage terms. Intermediate values of TF (617.7 mg kg^{-1}), and FDCs of WS-F and Res-F were found in Momela.

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~~ Table ~~4~~6.

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.

No significant correlations were found between any of the different soil fluoride fractions and the concentration of fluoride in plants tissues.

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~~WS-F 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 ~~5~~7).

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 ~~5~~7).

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 ~~1~~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~~4).

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 ~~2~~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.

Discussion

Distribution of ~~Soil water-soluble~~ fluoride fractions in the soil

The range of soil ~~water-soluble fluoride~~TF detected in our study is consistent with the values found by Dagnaw ~~et al~~*et al*. [42] in a study on the fluoride content of leafy vegetables in the Rift valley or elsewhere. The content of TF in soils is inherited from the minerals composing the bedrock and its distribution is related to pedogenic processes and soil texture [43]. However, as also observed in the present study, previous works that examined the fractional distribution of fluoride in soil identified the Res-F as a predominant fraction [17, 44, 45] normally existing in mineral form and hardly bioavailable[45]. ~~These~~ As regards to the WS-F, that together with the Ex-F is considered to form the labile and available soil fluoride pool [46], values measured in the study area ~~are~~ were from 2.7 to 5.5 times higher compared to those found by Dagnaw *et al.* [42] being also always significantly higher than the threshold of 16.4 mg kg^{-1} ~~in soil~~ of available fluoride in soil established by EPA, FAO, and WHO as cited by Lakshmi *et al.*[23], Limón-Pacheco *et al.* [38] and Paul *et al.* [39]. Soil ~~water-soluble fluoride~~WS-F is highly related to the soil type: factors such 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~~fluoride adsorption has been found to decrease with increasing ESP level or pH.[47]. In the present study, high soil ~~water-soluble fluoride~~WS-F and low Ex-F 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 [48]. Since –OH and F^- have almost the same diameter and compete for the same adsorption sites on the soil surface, at high pH, F^- may be displaced by –OH [17]. In addition, sandy soils, such as those of the study sites, have ~~the~~ a lower capability of F-retention than fine-textured soils because clay minerals containing Fe and Al can form very stable bonds with fluoride [3, 49]. Soil ~~Calcium~~ 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 [43, 50]. Concerning the OM-F, the observed values are consistent with the content of organic C of the soil, being the differences found between the three sites also related to this variable. Under certain soil conditions, the labile (WS-F and Ex-F) and the less or unavailable (OM-F, Fe/Mn-F and Res-F) forms of fluoride can be converted into each other [17]. Ws-F and Ex-F plant uptake processes can bring to a redistribution of the different fluoride forms in the soil in order to restore the chemical equilibrium [51]. Furthermore, the precipitation or dissolution of Fe/Mn oxides is associated with changes in soil redox condition, with consequent adsorption or release of the combined fluoride [52]. The Fe/Mn-F fraction represented, however, a very marginal fraction in the soils of the case study areas.

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

A positive relationship between the available fraction of soil fluoride and the content of fluoride in plants tissues were found in various studies conducted in controlled conditions [41, 53-55]. Differently, some other authors, according to the results observed in this study, found no significant correlation between the two variables in experiments at field scale [56, 57]. In addition, Stevens *et al.* [58] noted a wide range of variation in the correlation coefficients reported by different researchers (ranging from 0 to 0.78) [59]. Weinstein and Davison [59] hypothesised that this variability could be associated to: i) the desorption of fluoride due to the depletion caused by roots uptake, ii) the conditions of the extraction procedures, iii) the effect of soil pH on the equilibrium of the chemical species of fluoride in soil solution. Fluoride speciation, in fact, is considered a major factor controlling fluoride uptake. In acid soils, fluoride can form soluble complexes with aluminium, boron or silicon [50], while in alkaline soils, such as the ones considered in this study, the anion F^{-} is the predominant species [59]. However, the monitoring of the available fluoride dynamics during the whole crop cycle could be useful to better understand the relationship between soil and plant fluoride levels.

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 [20-23, 60], except for tomato in which roots and leaves fluoride contents were not significantly different, and for 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 [61], causing an imbalance both in biomass and fluoride partitioning in the different organs of the plant. In this regard, Kafkafi [62] reported that a reduction in bean root elongation, related to saline soils, was a factor preventing the ~~quantity~~ number 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~~*et al*. [21] and Gupta and Banerjee [63] on rice, Singh ~~et al~~*et al*. [64] on lady's finger and Yepu ~~et al~~*et al*. [41] on wheat. Ahmad ~~et al~~*et al*. [65] and Yadav ~~et al~~*et al*. [66] on mustard and Gautam and Bhardwaj [67] 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~~studied context [68, 69]. In addition, it was observed that an increase in soil ~~water-soluble fluoride~~WS-F corresponds to a general decrease in BCF values as reported by Zhang ~~et al~~*et al*. [51]. 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~~*et al*. [70] 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 alet al. [70] 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 Ciećko [55] 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 alet al [69], 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 alet al. [23] 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 alet al. [42] 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 [71].

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 [35]. As observed also by other authors in different contexts [69, 72, 73], Cchildren 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. Studies to establish the EDI of local populations with the related HI were also conducted in other contaminated areas of the world. Bhattacharya *et al.*[69] reported that in an area of West Bengal, more than 90% of the EDI derived from drinking water rather than from the food, whereas Brahman *et al.*[72] observed a significant contribution of the vegetables to the cumulative EDI in a population of Nagarparkar in India.

Conclusions

This study highlighted that ~~water-soluble fluoride~~WS-F content in agricultural soils of Ngarenanyuki area was greater than the limit of 16.44 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 mg kg^{-1} ~~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 mg kg^{-1} of dry matter ~~ppm~~ were observed for kale, tomato, bean and maize respectively, demonstrating that the considered food items, ~~that~~ which 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~~ available fluoride dynamics over the entire crop cycle and its relation with the contaminated irrigation ~~-~~waters ~~are worth~~also deserve further ~~investigations~~studies. Crops whose edible organs are roots, tubers and bulbs (e.g. carrots, potatoes, onion and turnip) are ~~also~~moreover worth to be investigated since they could possibly accumulate a higher amount of fluoride in edible parts. In addition, since in the rural areas of the EARV, crop residues of maize and bean are mainly used for feeding livestock, more research should be conducted to test whether the consumption of products (e.g. milk and meat) from animals fed with F-rich crop residues may represent an actual risk for human health.

Acknowledgements

This research was funded by the EU H2020 FLOWERED project 690378 “de - FLuoridation technologies for imprOving quality of WatEr and agRo - animal products along the East African Rift Valley in the context of aDaptation to climate change” coordinated by Prof. Giorgio Ghiglieri, University of Cagliari. The authors thank Emanuela Spanu and John Mshanga, for technical assistance.

References

1. Davies T, Environmental health impacts of East African Rift volcanism. *Environ Geochem Health* **30**:325-338 (2008).
2. Kloos H and Haimanot RT, Distribution of fluoride and fluorosis in Ethiopia and prospects for control. *Trop Med Int Health* **4**:355-364 (1999).
3. Edmunds WM and Smedley PL, Fluoride in natural waters, in *Essentials of Medical Geology: Revised Edition*, ed. by Selinus O. Springer, Dordrecht, pp 311-336 (2013).
4. Ghiglieri G, Pittalis D, Cerri G and Oggiano G, Hydrogeology and hydrogeochemistry of an alkaline volcanic area: the NE Mt. Meru slope (East African Rift–Northern Tanzania). *Hydrol Earth Syst Sci* **16**:529-541 (2012).
5. Ozsvath DL, Fluoride and environmental health: a review. *Rev Environ Sci Bio* **8**:59-79 (2009).
6. Frencken J, Endemic fluorosis in developing countries: causes, effects and possible solutions. Publ. No. 91.082. NIPG-TNO Institute for Preventive Health Care, Leiden (1992).
7. Kebede A, Retta N, Abuye C, Whiting S, Kassaw M, Zeru T, Tessema M and Kjellevold M, Dietary fluoride intake and associated skeletal and dental fluorosis in school age children in rural Ethiopian Rift Valley. *Int J Env Res Public Health* **13**:756 (2016).
8. Theiss FL, Couperthwaite SJ, Ayoko GA and Frost RL, A review of the removal of anions and oxyanions of the halogen elements from aqueous solution by layered double hydroxides. *J Colloid Interface Sci* **417**:356-368 (2014).
9. Lu Y, Sun Z, Wu L, Wang X, Lu W and Liu S, Effect of high-fluoride water on intelligence in children. *Fluoride* **33**:74-78 (2000).
10. Begum A, Harikrishna S and Khan I, Environmental fluoride level in Anekal Taluk of Bangalore District. *Rasayan j Chem* **1**:774-781 (2008).
11. Ali S, Thakur SK, Sarkar A and Shekhar S, Worldwide contamination of water by fluoride. *Environ Chem Lett* **14**:291-315 (2016).
12. Yoder KM, Mabelya L, Robison VA, Dunipace AJ, Brizendine EJ and Stookey GK, Severe dental fluorosis in a Tanzanian population consuming water with negligible fluoride concentration. *Community Dent Oral Epidemiol* **26**:382-393 (1998).
13. Vuhahula E, Masalu J, Mabelya L and Wandwi W, Dental fluorosis in Tanzania Great Rift Valley in relation to fluoride levels in water and in 'Magadi'(Trona). *Desalination* **248**:610-615 (2009).
14. Gupta S and Banerjee S, Fluoride Accumulation in Crops and Vegetables and Dietary Intake in a Fluoride-endemic Area of West Bengal. *Fluoride* **44**:153-157 (2011).
15. Pickering W, The mobility of soluble fluoride in soils. *Environ Pollut B* **9**:281-308 (1985).
16. Bustingorri C, Balestrasse K and Lavado RS, Effects of high arsenic and fluoride soil concentrations on soybean plants. *Phyton* **84**:407-416 (2015).
17. Gao H, Zhang Z and Wan X, Influences of charcoal and bamboo charcoal amendment on soil-fluoride fractions and bioaccumulation of fluoride in tea plants. *Environ Geochem Health* **34**:551-562 (2012).
18. Ahmad MA, Bibi H, Munir I, Ahmad MN, Zia A, Mustafa G, Ullah I and Khanc I, Fluoride toxicity and its effect on two varieties of *Solanum lycopersicum*. *Fluoride* **51**:267-277 (2018).
19. Bhargava D and Bhardwaj N, Phytotoxicity of fluoride on a wheat variety (*Triticum aestivum* var. Raj. 4083) and its bioaccumulation at the reproductive phase. *Asian J Exp Sci* **25**:37-40 (2011).
20. Takmaz-Nisancioglu S and Davison AW, Effects of Aluminium on Fluoride Uptake by Plants. *New Phytol* **109**:149-155 (1988).
21. Chakrabarti S, Patra PK and Mondal B, Uptake of fluoride by two paddy (*Oryza sativa* L.) varieties treated with fluoride-contaminated water. *Paddy Water Environ* **11**:619-623 (2013).
22. Baunthiyal M and Ranghar S, Physiological and Biochemical Responses of Plants under Fluoride Stress: An Overview. *Fluoride* **47**:287-293 (2014).

23. Lakshmi DV, Rao KJ, Ramprakash T, Reddy APK and Kendra KV, Bioaccumulation of fluoride in different plant parts of food crops grown in Narkatpally Mandal of Nalgonda District, Telangana. *Environ Ecol* **35**:1753-1758 (2017).

24. Oikos I, *The Mount Meru Challenge: Integrating conservation and development in Northern Tanzania*. Ancora Libri, Milan (2011).

25. Saxton KERWJ, Soil water characteristic estimates by texture and organic matter for hydrologic solutions, in *Soil Sci Soc Am J*, Ed, pp 1569-1578 (2006).

26. CLIMATE-DATA.ORG©, *Climate Olkung'wado, Climate Momella.* Available: https://en.climate-data.org/africa/tanzania/arusha-1666/ [15 March 2019].

27. Agrawala S, Moehner A, Hemp A, Aalst Mv, Hitz S, Smith J, Meena H, Mwakifwamba SM, Hyera T and Mwaipopo OU, Development and climate change in Tanzania: focus on Mount Kilimanjaro, in *COM/ENV/EPOC/DCD/DAC(2003)5/FINAL*, Ed. Organisation for Economic Co-operation and Development, Paris, pp 1-72 (2003).

28. Rizzu M, Akuno MH, Roggero PP, Wambu EW, Mtei KM and Seddaiu G, Characterizing cropping systems affected by fluoride contamination in eastern african countries in *Atti del XLVI Convegno della Società Italiana di Agronomia, Milano 2017*, Ed (2017).

29. Roser M and Ritchie H, Food per Person. *Our World in Data* (2019).

30. Berger T, Yu C, Drake H, Peltola P, Svensson D and Åström ME, Fluorine geochemistry of quaternary deposits in a nemo-boreal catchment with elevated dissolved fluoride in surface waters and groundwater. *J Geochem Explor* **170**:148-156 (2016).

31. Geretharan T, Jeyakumar P, Bretherton M and Anderson C, Defining a standard method to measure the total and bioavailable concentration of fluorine in New Zealand soils. *Microchem J* **142**:94-101 (2018).

32. Bustingorri C and Lavado RS, Soybean as affected by high concentrations of arsenic and fluoride in irrigation water in controlled conditions. *Agric Water Manage* **144**:134-139 (2014).

33. USEPA, Guidelines for exposure assessment, in *Federal Register Notice*, Ed by EPA, Washington, DC, pp 22887-22938 (1992).

34. ATSDR, Agency for Toxic Substances and Disease Registry. Report on toxicological profile for Fluorides, Hydrogen Fluoride, and Fluorine. U.S. Department of Health and Human Services. Public Health Service, Ed, Atlanta, GA (2003).

35. USEPA, Baseline Human Health Risk Assessment, Vasquez Boulevard and I-70 Superfund Site, Denver CO., Ed by Produced by USEPA R, with technical assistance from Syracuse Research Corporation., Denver, CO, pp 1-170 (2001).

36. USEPA, Framework for cumulative risk assessment, Ed by Risk Assessment Forum U, Washington, DC, pp 1-109 (2003).

37. FAO, Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. FAO, Food and Nutrition Technical Report Series 1, Ed, Rome (2001).

38. Limón-Pacheco JH, Jiménez-Córdova MI, Cárdenas-González M, Retana IMS, Gonsebatt ME and Del Razo LM, Potential Co-exposure to arsenic and fluoride and biomonitoring equivalents for Mexican children. *Ann Glob Health* **84**:257-273 (2018).

39. Paul E, Gimba C, Kagbu J, Ndukwe G and Okibe F, Spectrometric determination of fluoride in water, soil and vegetables from the precinct of river Basawa, Zaria, Nigeria. *J Basic Appl Chem* **1**:33-38 (2011).

40. R Core Team, R: A language and environment for statistical computing, Ed. R Foundation for Statistical Computing, Wien (2014).

41. Yepu L, ShengLi W, Prete D, SuYin X, ZhongRen N, Fei Z and Qian Z, Accumulation and interaction of fluoride and cadmium in the soil-wheat plant system from the wastewater irrigated soil of an oasis region in northwest China. *Sci Total Environ* **595**:344-351 (2017).

42. Dagnaw LA, Chandravanshi BS and Zewge F, Fluoride Content of Leafy Vegetables, Irrigation Water, and Farmland Soil in the Rift Valley and in Non-rift Valley Areas of Ethiopia. *Fluoride* **5**:409-429 (2017).

43. Kabata-Pendias A and Szteke B, Fluorine [F, 9], in *Trace Elements in Abiotic and Biotic Environments*. CRC press, Taylor & Francis Group, Boca Raton, pp 109-117 (2015).
44. Li Y, Wang S, Zhang Q, Zang F, Nan Z, Sun H, Huang W and Bao L, Accumulation, interaction and fractionation of fluoride and cadmium in sierozem and oilseed rape (*Brassica napus* L.) in northwest China. *Plant Physiol Biochem* **127**:457-468 (2018).
45. Yu L, Zhang J, Du C, Yang H and Ye B-C, Distribution and pollution evaluation of fluoride in a soil–water–plant system in Shihezi, Xinjiang, China. *Hum Ecol Risk Assess* **24**:445-455 (2018).
46. Yin J, Zheng Z, Li T, Zhang X, He S, Wang Y, Yu H and Liu T, Effect of tea plantation age on the distribution of fluoride and its fractions within soil aggregates in the hilly region of Western Sichuan, China. *J Soils Sed* **16**:2128-2137 (2016).
47. Chhabra R, Singh A and Abrol IP, Fluorine in sodic soils. *Soil Sci Soc Am J* **44**:33-36 (1980).
48. Choudhary S, Rani M, Devika OS, Patra A, Singh RK and Prasad SK, Impact of fluoride on agriculture: A review on it's sources, toxicity in plants and mitigation strategies. *Int J Chem Stud* **7**:1675-1680 (2019).
49. Omueti J and Jones R, Fluoride adsorption by Illinois soils. *J Soil Sci* **28**:564-572 (1977).
50. Kabata-Pendias A, Elements of group 17, in *Trace elements in soils and plants 4th Edition*. Taylor & Francis Group. CRC press., Boca Raton, pp 385-393 (2011).
51. Zhang J, Yu L, Yang H and Ye B-C, Migration and transformation of fluoride through fluoride-containing water for the irrigation of a soil–plant system. *Hum Ecol Risk Assess* **25**:1048-1058 (2018).
52. Zhang H, The Metropolis Pollution of Trace Metals—Shanghai and Nanjing, China, in *Behaviors of Trace Metals in Environment*, ed. by Springer, Singapore (2020).
53. Elrashidi MA, Persaud N and Baligar VC, Effect of fluoride and phosphate on yield and mineral composition of barley grown on three soils. *Commun Soil Sci Plant Anal* **29**:269-283 (1998).
54. Ruan JY and Wong MH, Accumulation of fluoride and aluminium related to different varieties of tea plant. *Environ Geochem Health* **23**:53-63 (2001).
55. Szostek R and Ciećko Z, Content of fluorine in biomass of crops depending on soil contamination by this element. *Fluoride* **47**:294-306 (2014).
56. Wang C, Yang Z, Chen L, Yuan X, Liao Q and Ji J, The transfer of fluorine in the soil-wheat system and the principal source of fluorine in wheat under actual field conditions. *Field Crops Res* **137**:163-169 (2012).
57. Jha SK, Nayak AK, Sharma YK, Mishra VK and Sharma DK, Fluoride Accumulation in Soil and Vegetation in the Vicinity of Brick Fields. *Bull Environ Contam Toxicol* **80**:369-373 (2008).
58. Stevens DP, McLaughlin MJ and Alston AM, Phytotoxicity of aluminium-fluoride complexes and their uptake from solution culture by *Avena sativa* and *Lycopersicon esculentum*. *Plant Soil* **192**:81-93 (1997).
59. Weinstein LH and Davison A, Uptake, Transport and Accumulation of Inorganic Fluorides by Plants and Animals, in *Fluorides in the Environment: Effects on Plants and Animals*. CABI Publishing, UK, pp 21-55 (2004).
60. Baunthiyal M and Ranghar S, Accumulation of Fluoride by Plants: Potential for Phytoremediation. *Clean-Soil Air Water* **43**:127-132 (2015).
61. Pessarakli M, Response of green beans (*Phaseolus vulgaris* L.) to salt stress, in *Handbook of Plant and Crop Stress, 2nd ed* ed. by Pessarakli M. Marcel Dekker, New York, pp 827-842 (1999).
62. Kafkafi U, Root growth under stress, in *Plant Roots: The Hidden Half*, ed. by Waisel Y, Eshel A and Kafkafi U. Marcel Dekker, New York, pp 375-391 (1991).
63. Gupta S and Banerjee S, Fluoride Accumulation in Paddy (*Oryza sativa*) Irrigated with Fluoride-contaminated Groundwater in an Endemic Area of the Birbhum District, West Bengal. *Fluoride* **42**:224-227 (2009).
64. Singh V, Gupta MK, Rajwanshi P, Mishra S, Srivastava S, Srivastava R, Srivastava MM, Prakash S and Dass S, Plant uptake of fluoride in irrigation water by ladyfinger (*Abelmorchus esculentus*). *Food Chem Toxicol* **33**:399-402 (1995).

65. Ahmad S, Saeed Ahmad S, Nauman Ahmad M, Ullah Shah R and Nawaz M, Bioaccumulation of fluoride ion at three flowering stages in different plant parts of mustard. *Fluoride* **48**:169-173 (2015).

66. Yadav M, Kumari N and Sharma V, Phytoremediation efficiency of *Brassica juncea* cultivars at vegetative and reproductive growth stages under individual and combined treatment of fluoride and aluminium. *Int J Phytoremediation* **20**:922-929 (2018).

67. Gautam R and Bhardwaj N, Bioaccumulation of Fluoride in Different Plant Parts of *Hordeum Vulgare* (barley) Var. Rd-2683 from Irrigation Water. *Fluoride* **43**:57-60 (2010).

68. Jha SK, Sharma YK, Damodaran T, Mishra VK and Sharma DK, Bio-concentration of fluoride in Lady's finger (*Abelmoschus esculentus*) grown in contaminated alkaline soil and evaluation of exposure risk in human. *Toxicol Environ Chem* **95**:138-149 (2013).

69. Bhattacharya P, Samal AC, Banerjee S, Pyne J and Santra SC, 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. *Environ Sci Pollut Res Int* **24**:20300–20314 (2017).

70. Nagaraju A, Thejaswi A and Aitkenhead-Peterson J, Fluoride and heavy metal accumulation by vegetation in the fluoride affected area of Talupula, Anantapur district, Andhra Pradesh. *J Geol Soc India* **89**:27-32 (2017).

71. Swartjes F, Dirven-van Breemen E, Otte P, van Beelen P, Rikken M, Tuinstra J, Spijker J and Lijzen J, Towards a protocol for the assessment of site-specific human health risks for consumption of vegetables from contaminated sites, Ed, Bilthoven, pp 1-130 (2007).

72. Brahman KD, Kazi TG, Baig JA, Afridi HI, Khan A, Arain SS and Arain MB, Fluoride and arsenic exposure through water and grain crops in Nagarparkar, Pakistan. *Chemosphere* **100**:182-189 (2014).

73. Kazi TG, Afridi HI, Baig JA and Brahman KD, Bioaccumulation of arsenic and fluoride in vegetables from growing media: health risk assessment among different age groups. *Environ Geochem Health* **41**:1223-1234 (2019).

74. McLean EO, Aluminum, in *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, Ed by G. NA. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, USA, pp 978-998 (1965).

75. Booty WG, Watershed acidification model and the soil acid neutralization capacity concept, Ed. McMaster University, Hamilton, Ont. Canada, p 194 (1983).

Figure legends

Figure 1. Concentrations of fluoride in the different soil F-fractions (Water Soluble F (WS-F), Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and residual F (Res-F)) in the topsoil (0-20 cm) of the three study sites (mean ± standard error (n = 12)).

Figure 12. 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 with increasing risks for human health.

Figure 23. 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 with increasing risks for human health.

Figure 34. Daily energy intake per kg of body weight by different ages, weights and lifestyle categories: a) children and adolescents, b) adults (rearranged from FAO[37]). 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.

Table 1 - 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 ~~(%)~~(g kg^{-1})							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~~ $cmol_{(+)}$ ~~100~~ kg^{-1})	11.7	14.6	12.6	20.6	11.0	14.7	ISO 11260
Exchangeable Ca ($cmol_{(+)}$ ~~meq 100~~ kg^{-1})	5.1	13.0	9.4	8.8	3.4	4.2	ISO 11260
Exchangeable Mg ($cmol_{(+)}$ ~~meq 100~~ kg^{-1})	0.8	0.8	1.8	1.6	0.3	0.5	ISO 11260
Exchangeable Na ($cmol_{(+)}$ ~~meq 100~~ kg^{-1})	1.8	1.7	4.3	6.5	6.7	5.9	ISO 11260
Exchangeable K ($cmol_{(+)}$ ~~meq 100~~ kg^{-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
Total Al (g kg^{-1})	38.3	40.6	39.5	40.1	36.8	35.6	EPA 3051 + GFAAS
Exchangeable Al (mg kg^{-1})	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	1.8	McLean [74] + GFAAS
Acid Neutralization Capacity ($cmol_{(+)}$ kg^{-1})	16.7	17.3	40.6	43.6	45.1	51.6	Booty [75] (0.01 M NaCl)

661 Table 2 - Plot size for each crop.

Crop	Plot size (m x m)	Space inter row (m)	Space between plants within row (m)	N. of plants per plot	N. of 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

662

663

Table 3 – Business as usual agricultural practices adopted for maize, tomato, bean and kale in the three study areas.

Agricultural practices	**Period**	**Specifications**	**Dose**
Land preparation	Pre-planting	Ploughing by hand hoe	-
Localized fertilization	One month after the planting	Urea (40% N) manually applied	10 g/plant
Weeding	Three times during the whole crop cycle at 2nd, 4th and 6th week after the planting	Manually by hand hoe	-
Insecticide application	Two weeks after the planting	Imidacloprid 17.1%	10mL/15L of water/plot
Fungicide application	Four weeks after the planting	Mancozeb 80%	80mL/15L of water/plot
	Six weeks after the planting	Mancozeb 80%	80mL/15L of water/plot

Table 4 - Soil ~~water~~Water ~~-soluble~~ Soluble ~~fluoride~~fluoride (WS-F) content in the 0-40 cm layer (mean ± standard error, n=12) in the three study sites.

Study site	**Soil ~~water-soluble F$^-$~~WS-F (~~WSF$^-$,~~ mg kg^{-1})**	**WS-F$^-$/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	*

† WS-F$^-$/ULF = Water-Soluble F$^-$/ limit of available fluoride in the soil
* = significant for $p \leq 0.05$; *** = significant for $p \leq 0.001$.

Table 5 - Fraction distribution coefficients (FDCs) of the different soil fluoride fractions for the three study sites: Water Soluble F (WS-F), Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and residual F (Res-F). FDCs are obtained by the ratio of each F fraction and the total F (%).

Soil fluoride fractions (%)	**Momela**	**Uwiro**	**Olkung'wado**
WS-F	10.8 Bb	20.3 Ab	4.9 Cb
Ex-F	0.5 Acd	0.6 Ad	0.3 Ac
OM-F	2.6 Bc	8.9 Ac	0.7 Bc
Fe/Mn- F	0.2 Ad	0.8 Ad	0.2 Ac
Res-F	86.0 Ba	69.4 Ca	94.0 Aa

† 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 soil fluoride fractions.

678 Table 6 - Fluoride concentration in maize, tomato, bean and kale plant tissues at the physiological
679 maturity stage of development (mg kg^{-1} of dry matter) and partitioning into plant parts for the three
680 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*

681 † Means followed by the same letter are not statistically different at $p \leq 0.05$; capital letters refer to comparisons among
682 sites, lower-case letters to plant parts.
683

Table 7 - 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 = (Translocation factor considering entire shoot system) F in entire shoot systems / F in roots
¶ TF EP = (Translocation factor considering edible part) F in edible part / F in roots

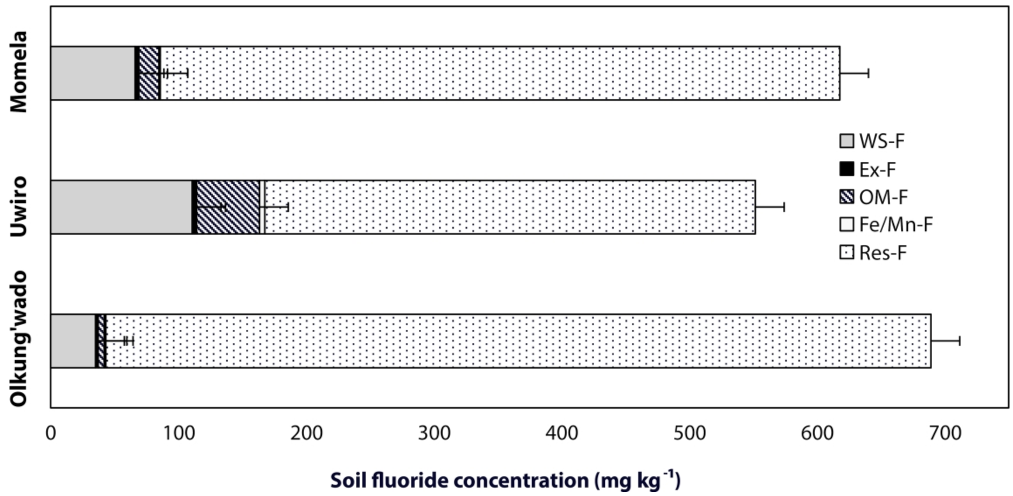

Figure 1. Concentrations of fluoride in the different soil F-fractions (Water Soluble F (WS-F), Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and residual F (Res-F)) in the topsoil (0-20 cm) of the three study sites (mean ± standard error (n = 12).

160x78mm (600 x 600 DPI)

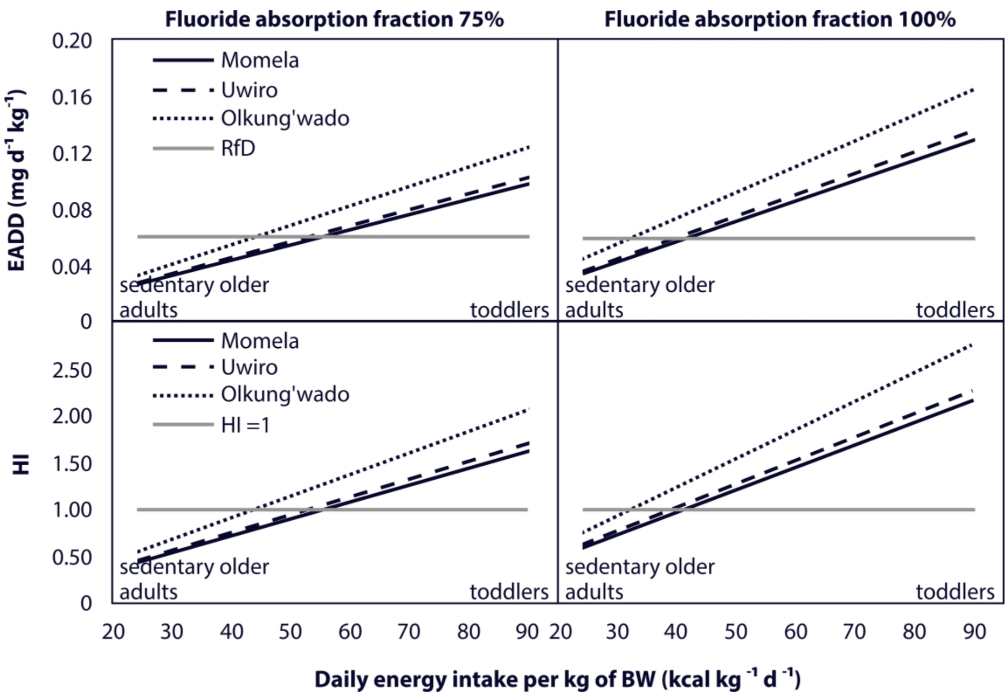

Figure 2. Cumulative EADD and HI, related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung’wado, at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human body (75% and 100%)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

149x102mm (600 x 600 DPI)

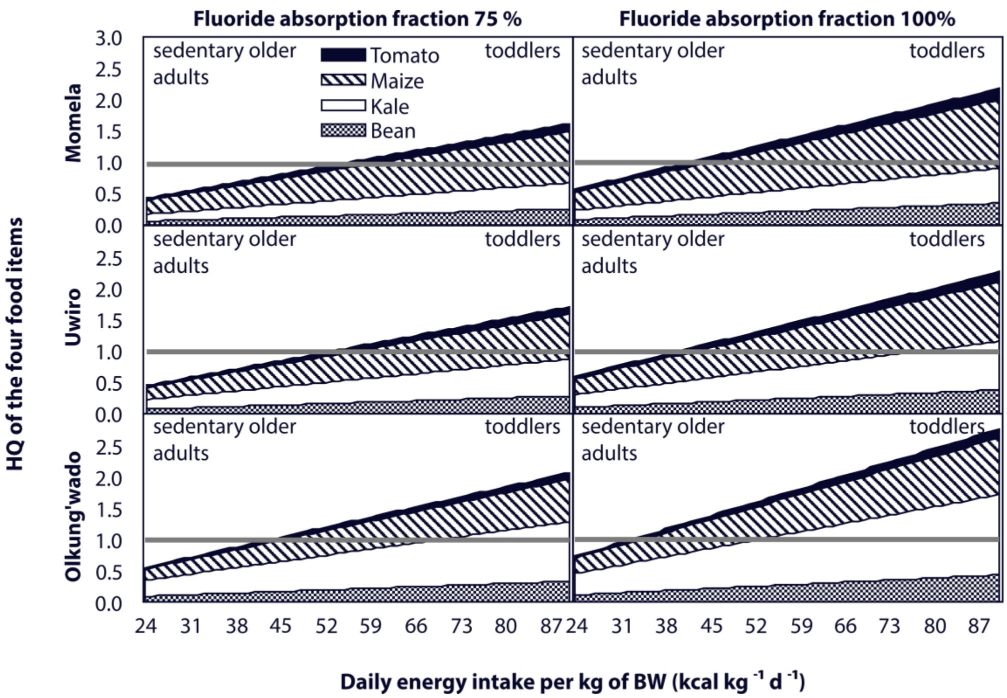

Figure 3. Contribution of each crop to the cumulative HI related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human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

156x106mm (600 x 600 DPI)

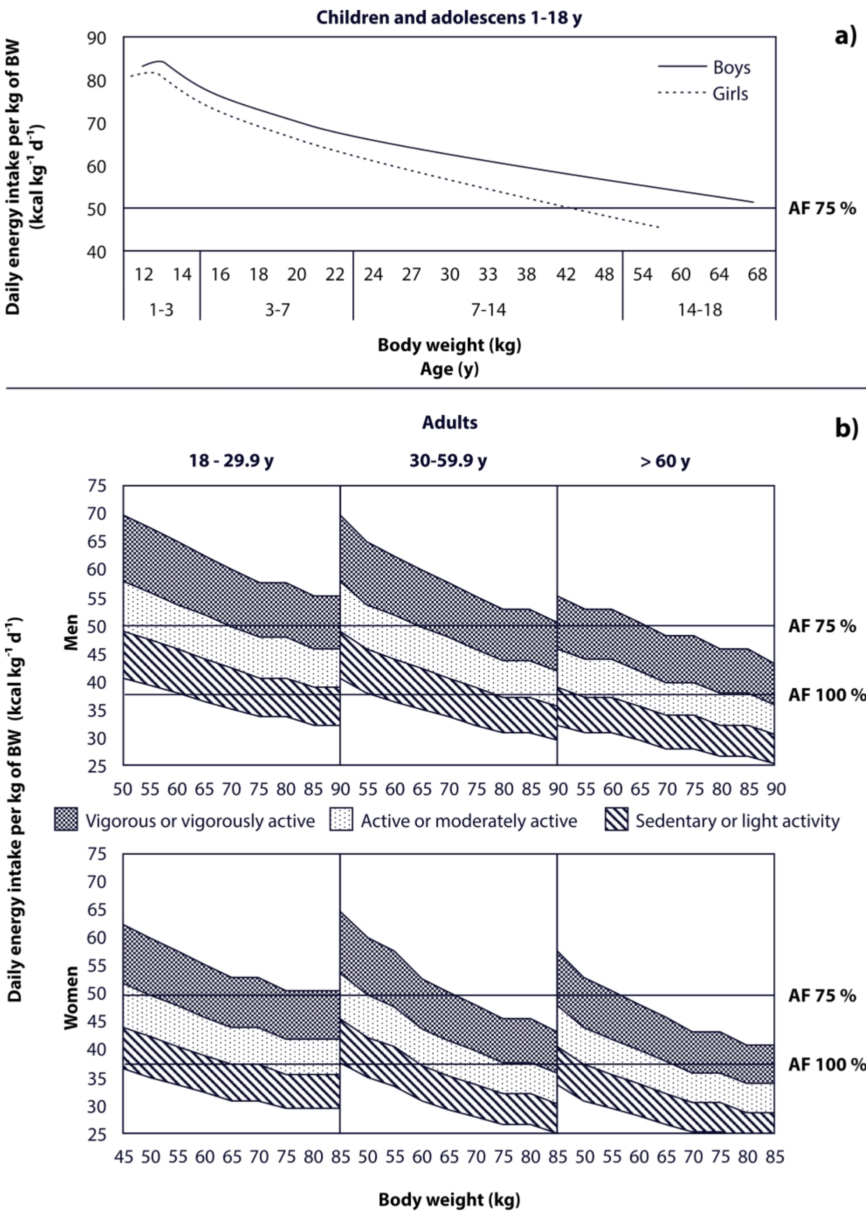

Figure 4. Daily energy intake per kg of body weight by different ages, weights and lifestyle categories: a) children and adolescents, b) adults (rearranged from FAO[37]). All the categories falling above the AF 75 % and AF 100 % lines correspond to the population groups with HI > 1 in the AF 75 % and AF 100 % scenarios respectively.

161x219mm (600 x 600 DPI)