

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

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## 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<sup>-1</sup> 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<sup>-1</sup> kg<sup>-1</sup> 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

## 37 Introduction

38 The East African Rift Valley (EARV) is among the few active rifts on the Earth's surface  
39 characterized by intense volcanic activity. Direct impacts of such volcanism include the rise, from  
40 the lower crust or upper mantle, of volatile and potentially harmful elements and their release to  
41 groundwater. These peculiar geological features have a great influence on the nature of the soils and  
42 the quality of water and food <sup>1</sup>. Particularly concerning fluorine (F), Kloos and Haimanot <sup>2</sup> reported  
43 that the concentrations of this element in the volcanic rocks of the East African Rift System are higher  
44 than those of the same kind of rocks in other regions. Some of the highest fluoride concentrations in  
45 groundwater ever recorded in the world were detected in the EARV <sup>3</sup>, such as 100 mg L<sup>-1</sup> or more  
46 measured in Tanzania by Ghiglieri *et al* <sup>4</sup>. In these areas, high concentrations of fluoride were  
47 also registered in surface water, up to 700 mg L<sup>-1</sup> in the Momela Lake. The Engare Nanyuki river,  
48 that is locally utilized as a source of irrigation since it is the only perennial stream in the East and  
49 North-East of Mount Meru, showed about 30 mg L<sup>-1</sup> of fluoride up to 60 mg F<sup>-</sup> L<sup>-1</sup> at its spring <sup>4</sup>.  
50 These concentrations can vary during the year due to different climatic conditions, as extreme  
51 evaporation has a consistent influence on the salt concentration.  
52 For over a hundred years, researchers from different disciplines studied the relationship between  
53 environmental fluoride contamination and health status of the population living in these so-called  
54 endemic areas <sup>5</sup>. Excessive and continuous intake of this element in the human diet can lead, in fact,  
55 to a series of severe diseases known as fluorosis <sup>6</sup>. The fluoride introduced with the diet is assimilated  
56 into teeth and bones <sup>7</sup> forcing out hydroxide ions from hydroxyapatite crystal with the formation of a  
57 tougher and stronger new mineral: fluorapatite <sup>8</sup>. Even if a small concentration (1.3 mg L<sup>-1</sup>) of fluoride  
58 intake is considered beneficial for the reduction of dental decay and the promotion of proper bone  
59 development, the ingestion of higher concentrations of fluoride can result into dental caries, mottled  
60 staining of teeth and malformations in bone structures <sup>1</sup>. Fluorosis can affect both young and old  
61 people. Fluoride ingestion during pregnancy and breastfeeding could affect the nervous system of the

62 developing foetuses and of the infants leading to future behavioural disorders and ~~reduced-low~~ IQ <sup>9</sup>,  
63 <sup>10</sup>.

64 Endemic fluorosis is a worldwide problem. Some countries such as Mexico are moderately affected  
65 while in other countries such as India, Tanzania, Ethiopia, Kenya and ~~Argentina~~Argentina, fluoride  
66 contamination in groundwater is a crucial matter <sup>11</sup>. In EARV about 90% of the population had shown  
67 fluorosis symptoms <sup>12</sup> and in the region of Arusha in Tanzania the prevalence of dental fluorosis is  
68 about 100% <sup>13</sup>. The Tanzanian national standard for drinking water has been raised up to 4 mg L<sup>-1</sup>  
69 respect to the WHO limit of 1.5 mg L<sup>-1</sup>, reflecting the difficult situation of both pollution of  
70 groundwater and water scarcity <sup>3</sup>.

71 Most health studies regard chronic fluoride exposure from drinking water. However, food can also  
72 be responsible for another potentially significant pathway <sup>5</sup>. Fluoride can be uptaken by food and feed  
73 crops through soil solution and gaseous emissions, entering the ~~man~~-food chain because of the  
74 consumption of contaminated vegetables and maybe also of livestock products about which very few  
75 data are however available in the scientific literature. Nevertheless, human health problems associated  
76 to the food chain are still poorly investigated, thus, the determination of fluoride intake from the daily  
77 diet is extremely valuable for enhancing our understanding of the role of food in fluorosis occurrence  
78 <sup>14</sup>.

79 The main natural source of fluoride in the agricultural soil is represented by the disruption of fluoride-  
80 rich minerals from the bedrock <sup>15</sup>. Other important sources are the irrigation water derived from  
81 groundwater and surface water as well as the surface runoff from contaminated sites to downstream  
82 areas <sup>16</sup>. Fluoride can then be detained in a durable way in soils and it is primarily associated with the  
83 colloid or clay fractions. For all soils, it is the soluble and exchangeable fluoride content which is  
84 considered is-biologically active for plants and animals while other fractions have been reported to  
85 have low or no bio-availability <sup>17</sup>. Plants roots passively absorb the ~~soluble-available~~ fluoride through  
86 the soil water solution and then it is moved via xylematic conducts up to the stems, leaves and fruits  
87 <sup>18</sup>. The bioaccumulation of fluoride can vary in the different plant parts, depending on both the transfer

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3 88 from soil solution to roots and the translocation from roots to shoots <sup>19</sup>, but many studies highlighted  
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5 89 a higher concentration of fluoride in roots than the other plant parts <sup>20-23</sup>.

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8 90 The aims of this study were (i) to assess the concentration of fluoride ~~content~~ in the edible portions  
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10 91 (leaves, fruits or seeds) of the main cultivated and consumed crops (bean, kale, maize, tomato) of the  
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12 92 study area in the Rift Valley of Northern Tanzania; (ii) to investigate the partitioning of fluoride  
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14 93 among the different organs of the plant in order to calculate the bioconcentration and translocation  
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16 94 factors.

## 17 95

### 18 96 **Materials and methods**

#### 19 97 **Study area**

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24 98 The study area is located in Northern Tanzania, in the Arusha Region, Arumeru District, within the  
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26 99 Ngarenanyuki ward (about 22'130 ha) <sup>24</sup>. Three different sites within this main region were identified  
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31 100 as representatives of the whole area mainly according to the willingness of the householder to  
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33 101 participate in the experiment and the reliability of the information provided: Uwiro (3°9'28.00"S,  
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35 102 36°51'23.10"E), Olkung'wado (3°11'20.42"S, 36°51'17.30"E) and Momela (3°12'54.50"S,  
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37 103 36°51'39.30"E).

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39  
40 104 The soil characteristics of the study sites are reported in Table 1. Soil textures vary from loamy sand  
41  
42 105 to sandy loam. Hydrological parameters were derived following Saxton <sup>25</sup> on the basis of the soil  
43  
44 106 texture, organic matter content and soil salinity. The soil pH varied from moderately alkaline to  
45  
46  
47 107 strongly alkaline. Regarding soil fertility, Uwiro was the site with the highest content of organic  
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49 108 carbon, total nitrogen and assimilable phosphates. Momela and Olkung'wado soils showed a low C/N  
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51 109 ratio and low Olsen P content. In addition, the organic C in Olkung'wado was poor. All experimental  
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54 110 sites showed an average CEC and a high to a very high content of exchangeable Na and K. Momela  
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56 111 and Uwiro were poor in exchangeable Ca while Olkung'wado was very poor. Exchangeable Mg was  
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58 112 poor in Momela, average in Uwiro and very poor in Olkung'wado. Mg/K ratio was very low for all  
59  
60 113 the three sites.

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3 114 Regarding salinity, Momela soil is considered non-saline, Uwiro slightly saline and Olkung'wado  
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5 115 from slightly to strongly saline. Considering the sodicity, soils were sodic in Uwiro and Olkung'wado  
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8 116 while normal in Momela.

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10 117 As regards the climatic features, the mean temperatures in the case study areas range from 11.6°C in  
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12 118 July to 27.7°C in February. The annual mean temperature is 18.9°C and the hottest and coldest season  
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15 119 fall between January and February and June to August respectively. With an Aridity Index (AI) of  
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17 120 0.63, the climate of the study area can be classified as dry-sub-humid <sup>26</sup>.

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19 121 Two main rainy periods characterize the rainfall pattern of the region. The first, between the ends of  
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21 122 February to mid-May, is known as “Masika” or long rainy period and is characterized by regular rains  
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24 123 (545 mm/year). The second one, in October-December, ~~present is characterized by~~ a higher variability  
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26 124 of rain intensity and distribution and it is named “Vuli” or short rainy season (210 mm/y<sup>-1</sup>). In the  
27  
28 125 rest of the year, dry periods prevail <sup>27</sup>. The investigation described in this work was conducted during  
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30  
31 126 the long rainy season from March to August 2018.

32  
33 127 The choice of the crops to be investigated was the outcome of 120 questionnaires collected locally  
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35 128 on cropping and livestock systems and dietary behaviour at household scale <sup>28</sup>. The survey highlighted  
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37 129 that most of the agricultural land in the three sites was cultivated with maize (*Zea mays* L.) as the  
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40 130 most common crop, followed by tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris*  
41  
42 131 L.) and an ecotype of kale (*Brassica sp. pl.*) locally known as “Sukuma wiki”. According to Roser  
43  
44 132 and Ritchie <sup>29</sup>, the average human daily energy requirement in Tanzania is 2208 kcal d<sup>-1</sup> per-capita  
45  
46  
47 133 (updated to 2013). With respect to this value, the average daily consumptions of the four considered  
48  
49 134 food items, as emerged from the survey, represent 26.3 %, 0.2 %, 6.8 % and 1.5 %, of the energy  
50  
51 135 consumption respectively.

### 136 **Experimental layout and crop management**

137 Every crop was replicated three times in each of the three selected sites within the study area, giving  
138 a total of 36 experimental units (3 sites x 4 crops x 3 replicates). Plot sizes for each crop are reported  
139 in Table 2.

140 In each of the three sites, maize and bean were manually seeded at the end of March 2018, while for  
141 tomato and kale a nursery using as substrate the soil of the corresponding area was prepared in the  
142 middle of April 2018 and the seedlings were transplanted after one month. Agricultural practices such  
143 as fertilization, weeding and application of pesticides were implemented according to similar business  
144 as usual practices in all the study sites by the local farmers; hence, since the experiment was conducted  
145 during the long rainy season, irrigation was not applied. ~~Localized fertilization was done after one~~  
146 ~~month from the seeding/transplanting with urea manually applied, following the business as usual~~  
147 ~~fertilization practices~~(Table 3).

### 149 **Plant sample collection**

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

### 154 **Chemical analyses**

155 For total F determination in plant tissues, powdered vegetable samples (0.2 g) were digested with 2  
156 ~~ml mL~~ of nitric acid ~~67%(15 mol L<sup>-1</sup>), 3 mL of~~ hydrogen peroxide ~~at 30%-(3-10 mol L<sup>-1</sup>)ml~~ and 5  
157 mL of deionised water ~~(5 ml)~~, in a microwave digestion unit (Milestone Ethos Easy, Milestone s.r.l,  
158 Sorisole (BG), Italy). In order to avoid losses of F in the form of HF, the vapour pressure of nitric  
159 acid was reduced immersing the still closed vessels in a refrigerated bath (-30 °C) for 30 min. The  
160 neutralization of digest solutions with aqueous NaOH (8 mol L<sup>-1</sup>, ~~M~~) was carried out directly inside

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2  
3 161 the vessels immediately after their opening. This procedure allowed to rapidly stop the leakage of  
4  
5 162 nitrous fumes and the consequent loss of analyte.

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8 163 For soil ~~water~~Water-soluble Soluble fluoride (WS-F) extraction, 2 g of sieved soil were shaken in  
9  
10 164 40 ~~ml~~mL of deionized water (ratio 1:20) for 16--18 h. The extraction fluid/sample mixture was  
11  
12 165 centrifuged and a 10 ~~ml~~mL aliquot from the supernatant was removed for the estimation of F.

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15 166 Different forms of F in soil (Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound  
16  
17 167 to organic matter (OM-F) and residual F (Res-F)) were determined by sequential extraction following  
18  
19 168 the method by Berger *et al.*<sup>30</sup>. Prior to the Ex-F extraction, the hot water-soluble F fraction was  
20  
21 169 removed (by shaking 0.5 h at 60°C; soil:solution ratio = 1:10) in order to discriminate the Ex-F from  
22  
23 170 the soluble fraction. Res-F was determined on the residue with the method advised by Geretharan *et*  
24  
25 171 *al.*<sup>31</sup>. The Total F was obtained by summing the different F fractions.

27  
28 172 Both F concentration of the plant samples digest solution and soil samples ~~water~~extraction solutions  
29  
30 173 were determined potentiometrically using a fluoride ion-selective electrode (ISE - Thermo Scientific  
31  
32 174 Orion 9609BNWP; measurement range: from  $10^{-6}$  mol L<sup>-1</sup> to a saturated F<sup>-</sup> solution). In order to adjust  
33  
34 175 ionic strength, buffer pH to 5.0-5.5, and break up metal-fluoride complexes, standards for the  
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36 176 instrument calibration and samples were mixed 1:9 with a total ionic strength adjustment buffer  
37  
38 177 (TISAB III) one hour prior to the determination.  
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## 44 179 **Data analyses**

46  
47 180 Bioconcentration *Factor-factor* (BCF) and translocation factor (TF)

48  
49 181 A common index for estimating the fluoride concentration in plants is the bioconcentration factor  
50  
51 182 (BCF) which is the ratio of fluoride concentration in the plant (or its component) and fluoride  
52  
53 183 concentration in soil. Another factor that focuses on the partitioning of fluoride in the various  
54  
55 184 vegetable organs and mainly in the translocation of fluoride within the plant is the translocation factor  
56  
57 185 (TF), explained as the ratio of fluoride concentration in the entire shoot system (ESS) or edible part  
58  
59 186 (EP) and the fluoride concentration in roots<sup>32</sup> :

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

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

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

191 For each study area, the individual fluoride exposure dose, coming from the four food items, was  
 192 estimated according to the following equation, readapted from <sup>33</sup>:

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

194 where C is the concentration of fluoride in maize/bean/tomato/kale in the different study areas (mg  
 195 kg<sup>-1</sup> DM), IR is the intake rate (kg d<sup>-1</sup>) and BW is the body weight (kg). AF is the absorption  
 196 fraction (dimensionless), i.e. the fraction of the ingested dose that is absorbed. Since the <sup>34</sup> reports  
 197 that fluoride is absorbed in a range between 75 % and 100 % during the digestion process, these two  
 198 limits were both assessed in two different scenarios.

199 The IR was estimated as follow:

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

201 where DCI is the Daily Calories Intake (kcal d<sup>-1</sup>) that is given by the DCI kg<sup>-1</sup> of BW multiplied by  
 202 the BW, FEC is the Food Energy Contribution of each considered food item over the DCI. On the  
 203 bases of the administered survey <sup>28</sup> the FEC was estimated to be 26.3 % for maize, 0.2 % for tomato,  
 204 6.8 % for bean and 1.5 % for kale. E represents the energy of each food item (kcal kg<sup>-1</sup>).

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

$$EADD_{food} = \frac{C \times DCI \text{ kg}^{-1} \times BW \times FEC \times AF}{BW \times E} = \frac{C \times DCI \text{ kg}^{-1} \times FEC \times AF}{E}$$

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

$$EADD_{cumulative} = EADD_{maize} + EADD_{bean} + EADD_{tomato} + EADD_{kale}$$

### 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<sup>36</sup> recommended a value of RfD for fluoride equals to, which is 0.06 mg kg<sup>-1</sup>.

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

$$HI = HQ_{maize} + HQ_{bean} + HQ_{tomato} + HQ_{kale}$$

Considering that IR depends on DCI kg<sup>-1</sup>, and in turns, DCI kg<sup>-1</sup> is related to the age, gender, weight and lifestyle of each individual, EADD and HI were reported by DCI kg<sup>-1</sup> values. DCI kg<sup>-1</sup> values for different stages of development, gender and lifestyle recommended by FAO<sup>37</sup> 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<sup>-1</sup><sup>23, 38, 39</sup> 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<sup>40</sup>. 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<sub>soil water-soluble fluoride</sub>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<sup>-1</sup>) established by EPA, FAO, and WHO<sup>23, 38, 39</sup> (Table 34).

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<sup>41</sup> (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<sup>-1</sup>), 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<sup>-1</sup>), 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<sup>-1</sup>), 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 46.

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257 Concerning maize and tomato, no significant interaction between sites and plant parts was found in  
258 terms of concentration of fluoride in the different plant parts (roots, leaves, stem and grains/fruits)  
259 following the same accumulation pattern in all the study sites: roots > leaves > stem > grains/fruits.  
260 In tomato, significant differences between sites were also observed with the highest fluoride plant  
261 accumulation in Olkung'wado and the lowest in Momela. A significant site x plant part interaction  
262 was observed for the fluoride plant uptake in bean. In Momela, the accumulation of fluoride was  
263 higher in roots compared to stem and seeds. In Olkung'wado, on the contrary, seeds accumulated  
264 more fluoride than roots and stem showed an intermediate value between roots and seeds. In Uwiro,  
265 no significant differences were observed in terms of fluoride accumulation in the three plant parts. In  
266 kale a significant site x plant part interaction was observed for the fluoride concentration in plants. In  
267 Momela and Uwiro the bioaccumulation of fluoride in kale plant tissues was highest in roots followed  
268 by leaves and stems that showed similar fluoride concentration. In Olkung'wado the fluoride  
269 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)**

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

277 The highest value of the BCF in the ESS was recorded in tomato, in Olkung'wado, while the lowest  
278 one in maize, in Uwiro. Regarding the BCF of EP the highest value was observed in Olkung'wado in  
279 the bean crop, and the lowest in maize, in Uwiro (Table 57).

280 The capability of plants to transfer fluoride from roots to the whole shoots or to the edible parts was  
281 evaluated through the calculation of the TF. The highest TF of EP was observed for bean (3.72) and  
282 the lowest for maize (0.19), both in the Olkung'wado experimental site while for the TF of ESS, the

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3 283 highest and the lowest values were 2.56 for bean and 0.23 for maize respectively, in Olkung'wado  
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5 284 and Uwiro sites (Table 57).

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## 12 287 **Exposure dose and health risk assessment**

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14 288 Cumulative fluoride exposure dose (EADD) and hazard index (HI) for each study area at two  
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17 289 hypothetical limit levels of fluoride absorption (AF) in the human body (75% and 100%) are shown  
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19 290 in Figure 12. The highest values were observed for a daily energy intake of 90 kcal d<sup>-1</sup> kg<sup>-1</sup> of BW,  
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21 291 that is the typical energy consumptions for kids at very earlier stages, while the lowest were associated  
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24 292 with values of 24 kcal d<sup>-1</sup> kg<sup>-1</sup> of BW, characteristics of elder people with a sedentary lifestyle (Figure  
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26 293 34).

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28 294 Considering a fluoride absorption of 75%, the EADD values ranged from 0.026 to 0.098 in Momela,  
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31 295 from 0.027 to 0.102 in Uwiro and from 0.033 to 0.124 mg F d<sup>-1</sup> kg<sup>-1</sup> of BW in Okung'wado. With the  
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33 296 100% F absorption scenario, the values of EADD ranged from 0.035 to 0.130 in Momela, from 0.036  
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35 297 to 0.136 and from 0.044 to 0.165 mg F d<sup>-1</sup> kg<sup>-1</sup> of BW in Okung'wado.

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37 298 Concerning HI, minimum values were 0.43, 0.45, 0.55 (AF 75%) and 0.58, 0.60, 0.73 (AF 100%) in  
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40 299 Momela, Uwiro and Olkung'wado respectively. Maximum values were 1.63, 1.70, 2.06 (AF 75%)  
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42 300 and 2.17, 2.27, 2.75 (AF 100%).

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44 301 The contribution of single hazard quotients (HQs) for each crop to the cumulative HI for each study  
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47 302 area are reported in Figure 23. In all the study sites, the main contribution to the HI was given by  
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49 303 maize crop, apart from Olkung'wado, in which kale played a major role.

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## 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-alet al.~~ <sup>42</sup> 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 <sup>43</sup>. 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 <sup>17, 44, 45</sup> normally existing in mineral form and hardly bioavailable <sup>45</sup>. These As regards to the WS-F, that together with the Ex-F is considered to form the labile and available soil fluoride pool <sup>46</sup>, values measured in the study area are-were from 2.7 to 5.5 times higher compared to those found by Dagnaw et al. <sup>42</sup> being also always significantly higher than the threshold of 16.4 mg kg<sup>-1</sup> ~~in soil~~ of available fluoride in soil established by EPA, FAO, and WHO as cited by Lakshmi et al. <sup>23</sup>, Limón-Pacheco et al. <sup>38</sup> and Paul et al. <sup>39</sup>. 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.~~ <sup>47</sup>. 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<sup>-</sup> concentration in the soil solution <sup>48</sup>. Since -OH and F<sup>-</sup> have almost the same diameter and compete for the same adsorption sites on the soil surface, at high pH, F<sup>-</sup> may be displaced by -OH <sup>17</sup>. 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 <sup>3, 49</sup>. 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<sub>2</sub>. On the contrary, a high presence of Na is related to high F solubility since NaF is

one of the most mobile compounds<sup>43, 50</sup>. 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<sup>17</sup>. 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<sup>51</sup>. 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<sup>52</sup>. 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<sup>41, 53-55</sup>. 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<sup>56, 57</sup>. In addition, Stevens *et al.*<sup>58</sup> noted a wide range of variation in the correlation coefficients reported by different researchers (ranging from 0 to 0.78)<sup>59</sup>. Weinstein and Davison<sup>59</sup> 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<sup>50</sup>, while in alkaline soils, such as the ones considered in this study, the anion F<sup>-</sup> is the predominant species<sup>59</sup>. 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.

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3 356 Similar to what ~~was~~ found by other authors in various plant species, roots were, in the majority of  
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5 357 cases, the organs were F accumulated more <sup>20-23, 60</sup>, except for tomato in which roots and leaves  
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8 358 fluoride contents were not significantly different, and ~~for~~ bean in Olkung'wado, where roots had  
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10 359 lower values of fluoride with respect to seeds. This peculiarity could be explained by the saline-sodic  
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12 360 nature of the soil of this area, which may have induced bean roots stunting <sup>61</sup>, causing an imbalance  
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15 361 both in biomass and fluoride partitioning in the different organs of the plant. In this regard, Kafkafi  
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17 362 <sup>62</sup> reported that a reduction in bean root elongation, related to saline soils, was a factor preventing the  
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19 363 ~~quantity-number~~ of ions that reach the roots by diffusion, in order to limit their plant uptake just at  
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22 364 the required quantities.

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24 365 In all the examined crops, the second plant organs for fluoride concentration level were the leaves.  
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26 366 This trend was observed also by Chakrabarti ~~et al~~ <sup>21</sup> and Gupta and Banerjee <sup>63</sup> on rice, Singh ~~et~~  
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28 367 ~~al~~ <sup>64</sup> on lady's finger and Yepu ~~et al~~ <sup>41</sup> on wheat. Ahmad ~~et al~~ <sup>65</sup> and Yadav ~~et al~~ <sup>66</sup>  
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30 368 <sup>66</sup> on mustard and Gautam and Bhardwaj <sup>67</sup> on barley found instead that grains accumulated more  
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33 369 fluoride than leaves.

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35 370 For all the considered species in each site, BCF values were  $< 1$ , indicating that none of them behaves  
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38 371 as hyperaccumulator of fluoride in the ~~study-areastudied context~~ <sup>68, 69</sup>. In addition, it was observed  
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40 372 that an increase in soil ~~water-soluble-fluoride~~ WS-F corresponds to a general decrease in BCF values  
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42 373 as reported by Zhang ~~et al~~ <sup>51</sup>. This trend could be interpreted as a defence mechanism countering  
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45 374 ~~the~~ high fluoride toxicity.

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47 375 In addition, TF values were always less than one in all crops, illustrating their lower accumulation in  
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49 376 shoot systems or edible parts than in roots. The only exception was represented by bean in  
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51 377 Olkung'wado since its peculiar minor accumulation in roots already described. In general, there are  
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54 378 only a few studies regarding fluoride partitioning into different plant parts and even less for the  
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56 379 species investigated in the present study. Nagaraju ~~et al~~ <sup>70</sup> detected higher fluoride concentration  
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58 380 in bean stems compared to that observed in the present work, despite a lower fluoride concentration  
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60 381 in soil, hence the BCF calculated on the basis of this data would be greater than the values obtained

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3 382 in the current study for beans. Fluoride concentration in roots and grains were not reported by  
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5 383 Nagaraju *et al.*<sup>70</sup> hence it was not possible to compare our results with TF values from this study.  
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8 384 The higher accumulation of fluoride in roots was confirmed by a study of Szostek and Ciec ko<sup>55</sup> who  
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10 385 found that roots had on average 6-fold more fluoride compared to aerial biomass. From their data, it  
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12 386 was possible to calculate the TF in ESS, which ranged from 0.11 to 0.26, while in our study TF in  
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14 387 ESS ranged from 0.23 to 0.38. Instead, TF EP values could not be calculated from the same study,  
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17 388 since no data were reported about the fluoride concentration in edible parts.  
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19 389 The analyses derived from the data collected on tomato by Bhattacharya *et al.*<sup>69</sup>, revealed an  
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21 390 average value of BCF in EP of 0.05, which was about 3 times lower than that observed in our case  
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24 391 (0.17). With respect to TF in EP, Lakshmi *et al.*<sup>23</sup> data revealed a mean value of 0.54, while our  
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26 392 findings indicate an average TF of 0.37.  
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28 393 Among the examined crops, kale showed a higher fluoride concentration in edible parts on average,  
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30 394 given that the comestible organs are the leaves. The values of BCF in EP in kale was 0.13 while from  
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33 395 the data reported by Dagnaw *et al.*<sup>42</sup> we calculated a BCF value of 0.24.  
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35 396 The differences between our study and the other studies cited above are not surprising, considering  
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37 397 that BCF values can vary among the same vegetable species depending on all the factors that influence  
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40 398 plant development such as the soil features and growth rate<sup>71</sup>.  
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#### 44 400 **Exposure dose and health risk assessment**

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46 401 The results of EADD and HI, calculated for the three different areas of Momela, Uwiro and  
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48 402 Olkung'wado, chosen as representatives for the study area, highlight that the main consumed crops  
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50 403 by local populations give a significant contribution to the individual daily fluoride exposure and to  
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52 404 the associated HI.  
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55 405 When a value of  $HI < 1$  is found, there is no significant risk of non-carcinogenic effects while for HI  
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57 406 values  $> 1$ , possible non-carcinogenic effects may occur, with increasing probability at higher HI  
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59 407 values<sup>35</sup>. As observed also by other authors in different contexts<sup>69, 72, 73</sup>, Cchildren in very early  
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3 408 stages are the most vulnerable to fluoride diseases from chronic exposure since, due to their high  
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5 409 daily energy requirement per kg of body weight; they are more susceptible to reach the highest daily  
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8 410 fluoride intake in proportion to their weight. Among adolescents, only girls from 14 to 18 years old  
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10 411 fall in the  $HI < 1$  range in the AF 75 % scenario. Concerning adult population groups considered in  
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12 412 this study, just a few categories fall in the  $HI < 1$  range with an AF 100 % hypothesis, sedentary adults  
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15 413 and, among women, also part of the moderately active group. In the AF 75 % scenario instead, most  
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17 414 of the categories fall in the  $HI < 1$  range, apart from very active lifestyle men and women and  
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19 415 moderately active young men. Studies to establish the EDI of local populations with the related HI  
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21 416 were also conducted in other contaminated areas of the world. Bhattacharya *et al.*<sup>69</sup> reported that in  
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24 417 an area of West Bengal, more than 90% of the EDI derived from drinking water rather than from the  
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26 418 food, whereas Brahman *et al.*<sup>72</sup> observed a significant contribution of the vegetables to the cumulative  
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28 419 EDI in a population of Nagarparkar in India.

## 33 421 Conclusions

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35 422 This study highlighted that ~~water-soluble fluoride~~ WS-F content in agricultural soils of Ngarenanyuki  
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37 423 area was greater than the limit of  $16.44 \text{ mg kg}^{-1}$  as established by EPA, FAO, and WHO Joint  
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40 424 Standards, with an average of  $63.7 \pm 2.5$ ,  $133.1 \pm 9.0$ ,  $129.6 \pm 52.7 \text{ mg kg}^{-1}$  ~~ppm~~ in Momela, Uwiro  
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42 425 and Olkung'wado subareas respectively.

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44 426 Despite all the studied crops showed  $BCF < 1$  values, indicating that none of them is  
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47 427 hyperaccumulator in the study area, fluoride movement from contaminated soils to food crops was  
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49 428 reflected in a substantial accumulation of fluoride in plants edible parts. Average concentrations of  
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51 429  $14.2$ ,  $11.4$ ,  $11.3$  and  $8.0 \text{ mg kg}^{-1}$  ~~of dry matter ppm~~ were observed for kale, tomato, bean and maize  
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54 430 respectively, demonstrating that the considered food items, ~~that-which~~ are among the most consumed  
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56 431 in the rural area under study, substantially contribute to fluoride-correlated diseases, especially in  
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58 432 earlier ages, also without considering the contribution of the drinking water. For children and  
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60 433 adolescents, a high risk of non-carcinogenic effects from fluoride exposure was highlighted, for both

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3 434 AF 75 % and AF 100 % hypothesis, whereas regarding the adult population groups a big difference  
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5 435 emerged among AF 75 and AF 100 % scenarios, with a wider range of under-risk people in the latter  
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8 436 case.

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10 437 The results obtained in this study are only referred to crops grown in the EARV in the wet season,  
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12 438 thus, further experiments are needed to assess the bioaccumulation of fluoride in food crops during  
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15 439 the dry season, when F-rich irrigation water is employed to satisfy crop water requirements.

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17 440 Furthermore, the soil ~~soluble-available~~ fluoride dynamics over the entire crop cycle and its relation  
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19 441 with the contaminated irrigation ~~-waters~~ are worth also deserve further ~~investigations studies~~. Crops  
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22 442 whose edible organs are roots, tubers and bulbs (e.g. carrots, potatoes, onion and turnip) are  
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24 443 ~~also moreover~~ worth to be investigated since they could possibly accumulate a higher amount of  
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26 444 fluoride in edible parts. In addition, since in the rural areas of the EARV, crop residues of maize and  
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28 445 bean are mainly used for feeding livestock, more research should be conducted to test whether the  
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31 446 consumption of products (e.g. milk and meat) from animals fed with F-rich crop residues may  
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33 447 represent an actual risk for human health.

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49 454 assistance.

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3 641 **Figure legends**  
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5 642 Figure 1. Concentrations of fluoride in the different soil F-fractions (Water Soluble F (WS-F),  
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8 643 Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and  
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10 644 residual F (Res-F)) in the topsoil (0-20 cm) of the three study sites (mean  $\pm$  standard error (n = 12)).

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12 645 **Figure 12.** Cumulative EADD and HI, related to the consumption of the four investigated food items  
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14 646 cultivated in the study areas of Momela, Uwiro and Olkung'wado, at increasing levels of daily energy  
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17 647 intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human body (75% and  
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19 648 100%) were assessed. RfD is the reference dose for humans associated with the 'no adverse effect  
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22 649 level'. HI > 1 is associated ~~to~~-with increasing risks for human health.

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24 650 **Figure 23.** Contribution of each crop to the cumulative HI related to the consumption of the four  
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26 651 investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado at  
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29 652 increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride  
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31 653 absorption in human body (75% and 100%) were assessed. HI > 1 is associated ~~to~~-with increasing  
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33 654 risks for human health.

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35 655 **Figure 34.** Daily energy intake per kg of body weight by different ages, weights and lifestyle  
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38 656 categories: a) children and adolescents, b) adults (rearranged from FAO<sup>37</sup>). All the categories falling  
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40 657 above the AF 75 % and AF 100 % lines correspond to the population groups with HI > 1 in the AF  
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42 658 75 % and AF 100 % scenarios respectively.  
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659 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
<i>Depth (cm)</i>	<i>0-20</i>	<i>20-40</i>	<i>0-20</i>	<i>20-40</i>	<i>0-20</i>	<i>20-40</i>	
Particle size classes (%) (g kg <sup>-1</sup> )							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 <sup>-1</sup> )	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 <sup>-1</sup> )	11.7	4.0	24.7	17.7	5.9	3.9	ISO 10694
Total N (g kg <sup>-1</sup> )	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 <sub>2</sub> O <sub>5</sub> , mg kg <sup>-1</sup> )	9.2	9.2	128.3	96.3	9.2	18.4	Method XIV.1 G.U. 248 21/10/1999
CEC (meq cmol <sub>(+)</sub> 100 kg <sup>-1</sup> )	11.7	14.6	12.6	20.6	11.0	14.7	ISO 11260
Exchangeable Ca (cmol <sub>(+)</sub> meq 100 kg <sup>-1</sup> )	5.1	13.0	9.4	8.8	3.4	4.2	ISO 11260
Exchangeable Mg (cmol <sub>(+)</sub> meq 100 kg <sup>-1</sup> )	0.8	0.8	1.8	1.6	0.3	0.5	ISO 11260
Exchangeable Na (cmol <sub>(+)</sub> meq 100 kg <sup>-1</sup> )	1.8	1.7	4.3	6.5	6.7	5.9	ISO 11260
Exchangeable K (cmol <sub>(+)</sub> meq 100 kg <sup>-1</sup> )	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 <sup>-1</sup> 25 °C	0.8	0.7	3.1	3.1	2.8	9.7	ISO 11265
Soluble Ca in H <sub>2</sub> O (1:5) (mg kg <sup>-1</sup> )	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 <sub>2</sub> O (1:5) (mg kg <sup>-1</sup> )	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 <sub>2</sub> O (1:5) (mg kg <sup>-1</sup> )	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 <sub>2</sub> O (1:5) (mg kg <sup>-1</sup> )	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 <sup>-1</sup> )	<u>38.3</u>	<u>40.6</u>	<u>39.5</u>	<u>40.1</u>	<u>36.8</u>	<u>35.6</u>	<u>EPA 3051 + GFAAS</u>
Exchangeable Al (mg kg <sup>-1</sup> )	<u>&lt; 0.15</u>	<u>&lt; 0.15</u>	<u>&lt; 0.15</u>	<u>&lt; 0.15</u>	<u>&lt; 0.15</u>	<u>1.8</u>	<u>McLean<sup>74</sup> + GFAAS</u>
Acid Neutralization Capacity (cmol <sub>(+)</sub> kg <sup>-1</sup> )	<u>16.7</u>	<u>17.3</u>	<u>40.6</u>	<u>43.6</u>	<u>45.1</u>	<u>51.6</u>	<u>Booty<sup>75</sup> (0.01 M NaCl)</u>

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 <sup>-2</sup>
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

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

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

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

Study site	Soil <del>water-soluble F</del> <del>WS-F</del> (WSF, mg kg <sup>-1</sup> )	WS-F/ULF <sup>†</sup>	Significance of t-test
Momela	63.7 $\pm$ 2.5	3.9	***
Uwiro	133.1 $\pm$ 9.0	8.1	***
Olkung'wado	129.6 $\pm$ 52.7	7.9	*

<sup>†</sup> WS-F/ULF = Water-Soluble F/ limit of available fluoride in the soil

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

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3 671 Table 5 - Fraction distribution coefficients (FDCs) of the different soil fluoride fractions for the three  
4 672 study sites: Water Soluble F (WS-F), Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F),  
5 673 F bound to organic matter (OM-F) and residual F (Res-F). FDCs are obtained by the ratio of each F  
6 674 fraction and the total F (%).

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

15 675 † Means followed by the same letter are not statistically different at  $p \leq 0.05$ ; capital letters refer to comparisons among  
16 676 sites, lower-case letters to soil fluoride fractions.  
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Table 6 - Fluoride concentration in maize, tomato, bean and kale plant tissues at the physiological maturity stage of development (mg kg<sup>-1</sup> 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 <i>d</i>
	Leaves	25.8	24.3	23.8	24.6 <i>b</i>
	Stem	15.0	10.6	16.0	13.9 <i>c</i>
	Roots	37.3	38.6	38.1	38.0 <i>a</i>
	Mean	21.7 <i>A</i>	20.3 <i>A</i>	21.31 <i>A</i>	21.1
Tomato	Fruits	12.9	11.4	10.3	11.4 <i>c</i>
	Leaves	33.8	37.3	40.3	37.6 <i>a</i>
	Stem	10.4	17.1	25.6	18.6 <i>b</i>
	Roots	29.4	35.1	35.4	33.8 <i>a</i>
	Mean	21.6 <i>B</i>	25.3 <i>AB</i>	27.9 <i>A</i>	25.3
Bean	Seeds	10.4 <i>Ab</i>	11.3 <i>Aa</i>	12.7 <i>Aa</i>	11.3
	Stem	4.0 <i>Ab</i>	8.5 <i>Aa</i>	6.9 <i>Aab</i>	6.4
	Roots	23.3 <i>Aa</i>	16.3 <i>Aa</i>	3.4 <i>Bb</i>	15.7
	Mean	12.6	12.0	7.7	11.1
Kale	Leaves	9.0 <i>Bb</i>	12.7 <i>Bb</i>	20.9 <i>Ab</i>	14.2
	Stem	7.5 <i>Ab</i>	5.0 <i>Ab</i>	3.6 <i>Ac</i>	5.3
	Roots	23.6 <i>Ba</i>	33.7 <i>Aa</i>	37.9 <i>Aa</i>	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.

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684 Table 7 - Bioconcentration (BCF) and translocation (TF) factors calculated for the investigated crops  
685 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

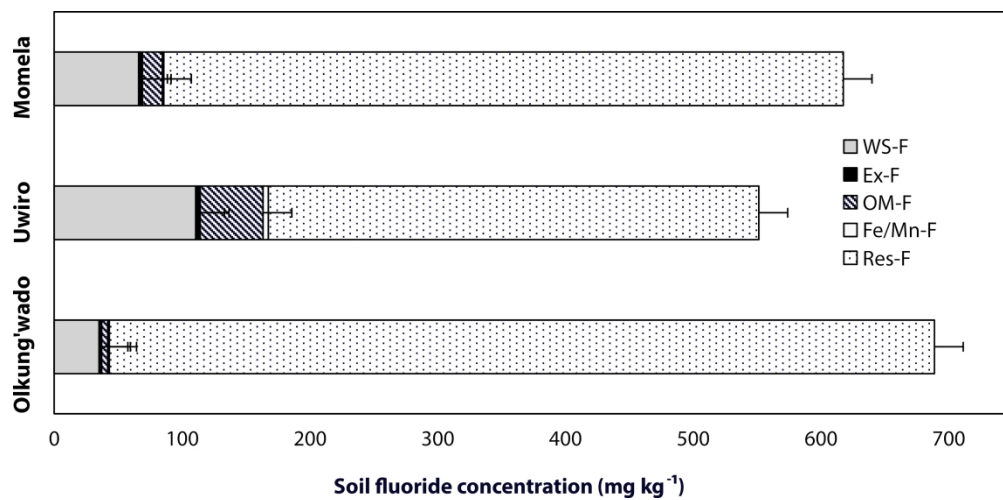


Figure 1. Concentrations of fluoride in the different soil F-fractions (Water Soluble F (WS-F), Exchangeable F (Ex-F), F bound to Fe/Mn oxides (Fe/Mn-F), F bound to organic matter (OM-F) and residual F (Res-F)) in the topsoil (0-20 cm) of the three study sites (mean  $\pm$  standard error (n = 12)).

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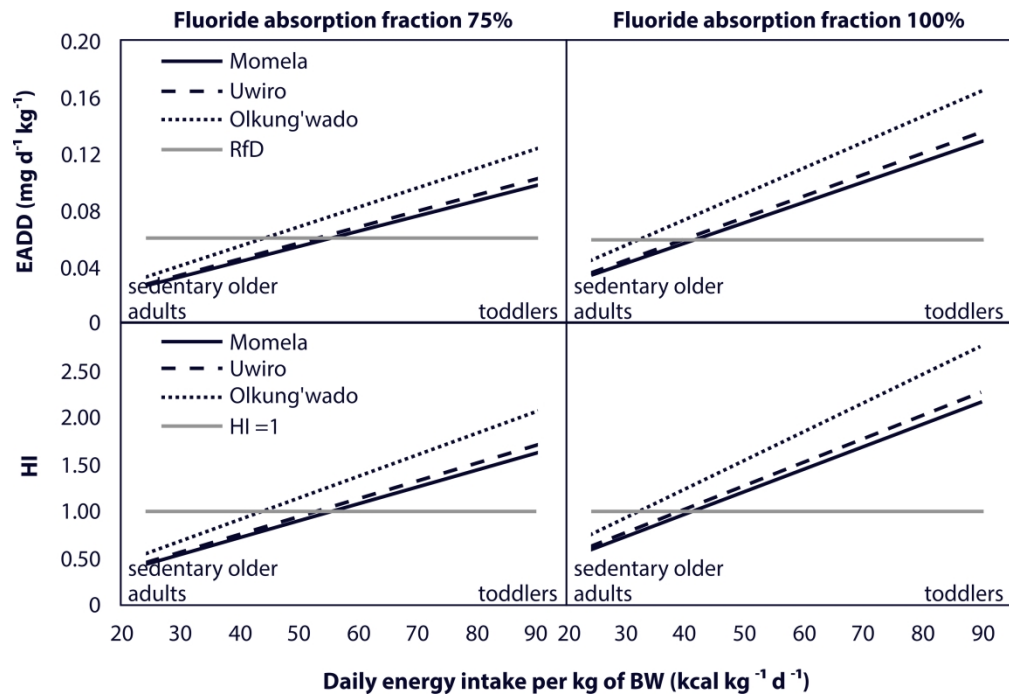


Figure 2. Cumulative EADD and HI, related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado, at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human body (75% and 100%) 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.

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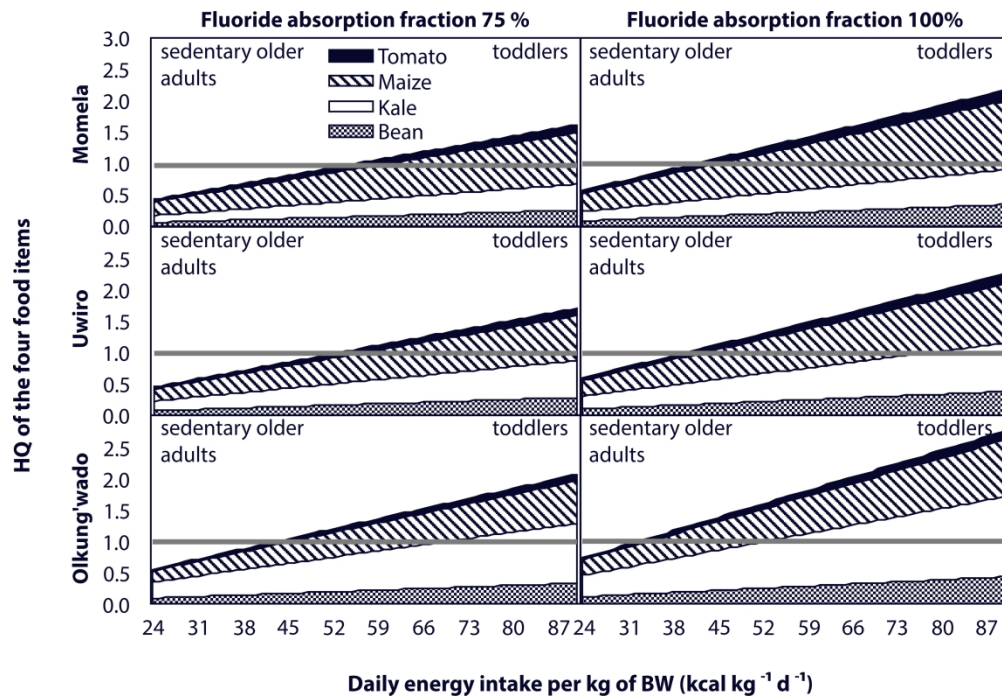


Figure 3. Contribution of each crop to the cumulative HI related to the consumption of the four investigated food items cultivated in the study areas of Momela, Uwiro and Olkung'wado at increasing levels of daily energy intake per kg of BW. Two hypothetical limit levels of fluoride absorption in human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

156x106mm (600 x 600 DPI)

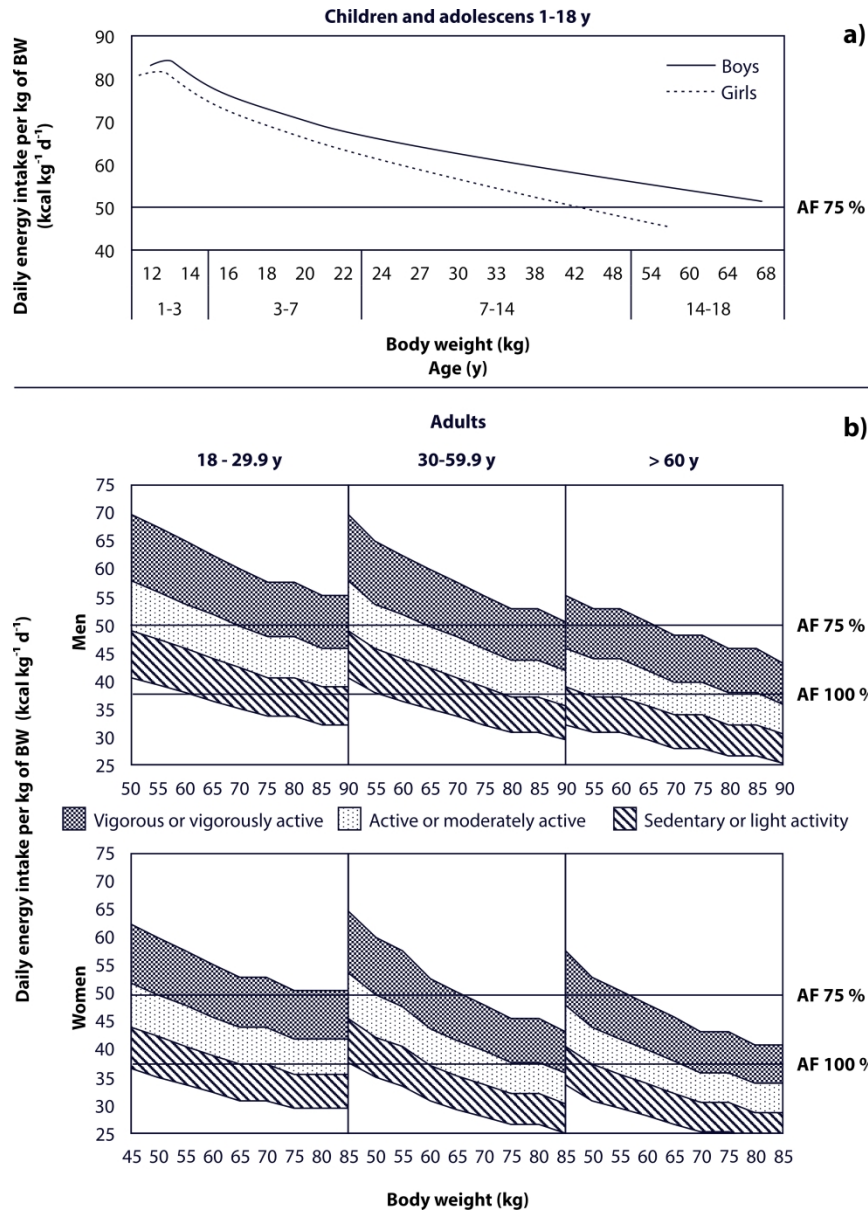


Figure 4. Daily energy intake per kg of body weight by different ages, weights and lifestyle categories: a) children and adolescents, b) adults (rearranged from FAO<sup>37</sup>). All the categories falling above the AF 75 % and AF 100 % lines correspond to the population groups with HI > 1 in the AF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

161x219mm (600 x 600 DPI)