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RESEARCH ARTICLE

Metal Accumulation in *Amaranthus cruentus* Cultivated on Different Systems of Tropical Urban Gardens

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Abstract

Improving of agricultural yields is basic for assessment and efficient management of soil and crop systems. After investigation of the soil proprieties influenced by urban gardening in Libreville, the present study aims at assessing soil-plant transfer of metals. Amaranth was harvested in urban gardens under shelter and in open field. The concentrations of metals in soils, leaves and roots were determined by ICP-AES after acidic mineralization. Soil physico-chemical properties and metal concentrations, cropping system and time of cultivation seemed to have impact on metal uptake by plant. Al, Mn and Zn were significantly more bioconcentrated in Amaranth grown under protective structure than Amaranth grown in open field, while Fe in roots and Cu were significantly more bioconcentrated in Amaranth from open field than Amaranth cultivated under shelter. The bioconcentration factor of Zn in all samples was >1 confirming that Amaranth could be used as food crop for the supplementation of Zn in the case of deficiency in this metal. But the high concentration of Al in leaves of Amaranth cultivated on these tropical soils suggests a toxicity risk of this metal in food chain. The findings indicate that Cu, Mn and Zn accumulated by Amaranth grown on all soils, and Fe accumulated by Amaranth under shelter were largely translocated in the leaves. The translocation of metals in plants was influenced by the soil metal and agricultural system concerned.

Keywords: Agricultural yields, Libreville, Amaranth, urban gardens, metal uptake, translocation.

Introduction

Plants need essential metals for normal functioning of their metabolism, but other non-essential metals are taken up by plants as well and in excess, essential and non-essential metals are absorbed by roots and induce deleterious effects on various physiological processes such as movement in xylem, movement in phloem, storage, accumulation and immobilization (Kabata-Pendias, 2011). The chelating ligands are important in the control of cation translocation in plants. However, other factors such as pH, competing cations, and formation of insoluble salts (phosphate, oxalate, etc.) govern metal mobility within plant tissues. The complex of cations with organic acids (e.g., citric, malic and amino acids) prevents their immobilization in the xylem and allows their transfer to the shoots. The immobilization of metals in roots, due to various processes, has a dominating impact on their translocation to the above-ground parts (Kabata-Pendias, 2011). The accumulation of metals in edible parts of crops can have both positive and negative health effects. Some metallic elements such as aluminum (Al), cadmium (Cd) and lead (Pb) can be toxic to plants, animals and humans (Terzano *et al.,* 2008). In addition, others elements such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) are essential for growth and life of plants, animals and humans, but insufficient or high levels of essential elements cause serious human, animal and plant disease (Young-Eun *et al.,* 2007). Vegetables hold an important place in well-balanced diets and increasing consumption of vegetable and fruits is advisable (Lu *et al.,* 2003). For e.g., *Amaranthus cruentus* is a leafy vegetable with high nutritional value grown in all seasons, produced and consumed in South America, Asia and Africa. The Amaranth leaves can be processed into many food products. They contain high levels of vitamin A, calcium and potassium (Akanbi and Togun, 2002). Some studies have showed that Amaranth could have a good accumulation capacity of metals (Ondo *et al.,* 2013a). After a previous study on characterization of gardening soils in urban area of Libreville (Ondo *et al.,* 2013b), this study thus, aimed at assessing the concentration of metals (Al, Cu, Fe, Mn and Zn) in Amaranth cultivated in these urban gardens as function of agricultural practices and cultivation time.

Materials and methods

Study site: This study was carried out in 2008 and 2009 in urban garden areas of Libreville, capital of Gabon (9°25' East and 0°27' North).

The climate is hot and humid with two rainy seasons and two dry seasons. It is characterized by an average rate of annual rainfall that varies from 1,600 to 1,800 mm. Hygrometry is usually above 80% and reaches 100% during the rain seasons. Monthly-averaged temperatures oscillate between 25 and 28°C, with daily minima (18°C) in July and maxima (35°C) in April.

Sampling and sample preparation: Eleven plots on nine sites (PRE, NTO, CHA, IPH, BAS, ALI, MEL, SIB and CAM) were selected for this study (Ondo *et al.,* 2013b). The major distinctions between the plots were due to cultivation periods (between 2 and 38 years) and operating system: gardens cultivated in open field (OF) and gardens cultivated under shelter (PS). The study plots were separated according to time of exploitation (urban gardens cultivated for <10 years and those cultivated since 10 years and more) and cultural practices (OF and PS). This led to define four groups of plots: gardens cultivated under shelter (PS) for <10 years (PS1) or >10 years (PS2), gardens cultivated in open field (OF) for <10 years (OF1) or >10 years (OF2). On each plot, the samples of vegetables (*A. cruentus*) were collected in the same areas of soil sampling. It was pay attention that each vegetable samples had reached the same degree of maturation. Roots and aerial parts (leaves, the edible part) were collected with at least three replications by plots. Plant material was carefully washed with de-ionized and further, ultra-pure water to remove dust and soil particles, and then air-dried and stove at 70°C until constant weight.

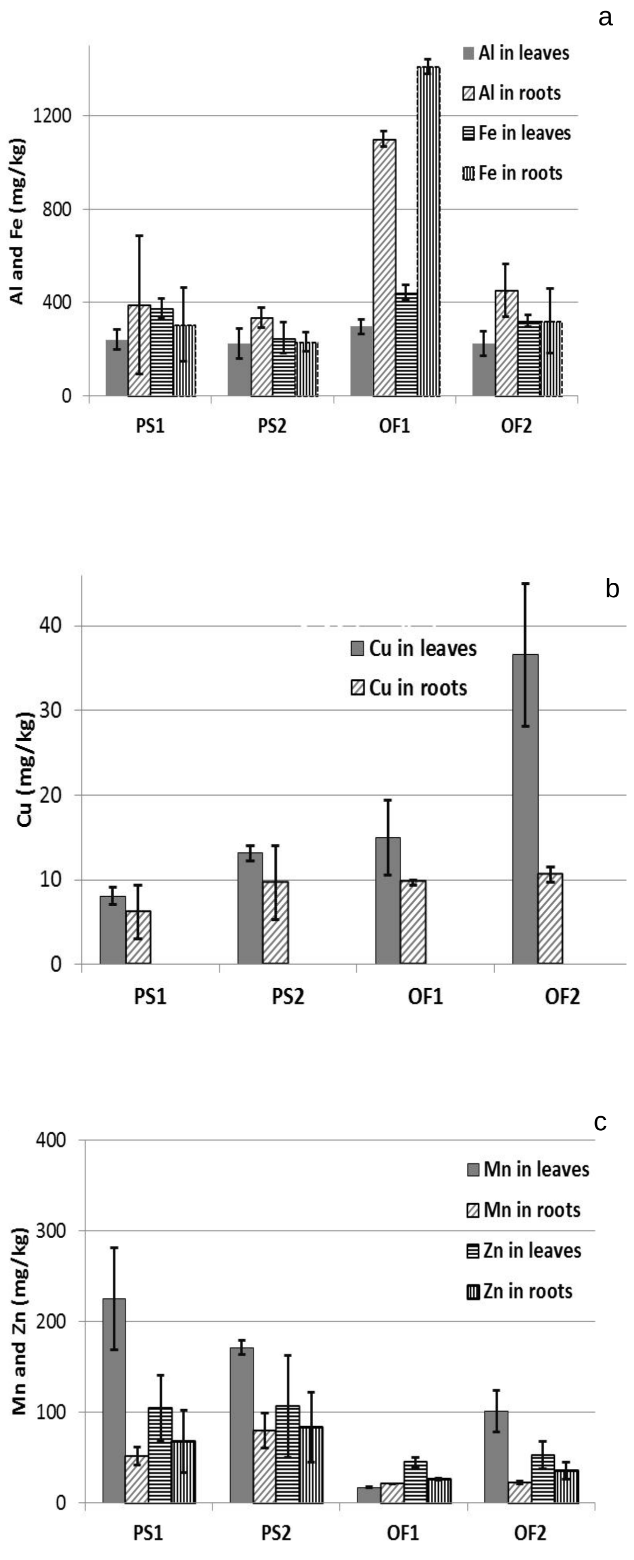
Analysis of metal concentrations in plants: Plant samples were digested for 1 h at 150°C in a microwave mineralizer, using a mixture of nitric acid, hydrogen peroxide and ultra-pure water with a volume ratio of 2:1:1 (Nardi *et al.,* 2009). The resulting solution was then filtered at 0.45 µm and stored at 4°C before the Inductively Coupled Plasma Atomic Emission Spectroscopy ICP-AES (Jobin Yvon Horiba, Spectra 2000) analysis was done in order to determine metal concentrations.

Statistical analysis: The mean, standard error and mean comparison tests were carried out. Correlation matrix was used to identify the relationship between heavy metal contents in soils and parts of Amaranth. Multivariate analysis methods such as Principal Component Analysis (PCA) and two-way analysis of variance (ANOVA) were used to extract the information from soil properties and metals in plants. The statistical analyses were performed using XLSTAT 2010, 6.04 version.

Results and discussion

Metals in Amaranth: Some differences in metal levels could be observed between roots and leaves of Amaranth (Fig. 1).

Fig. 1a-c. Metals concentrations (Al, Fe, Cu, Mn and Zn) in roots and leaves of Amaranth (mg/kg, dry weight) cultivated in urban gardens of Libreville. PS: gardens under shelter, OF: gardens in open field, 1: exploited since <10 years and 2: exploited during >10 years.



So, significant heterogeneity was noted inside same sub-group for manganese in the Amaranth leaves of PS1 sites, copper in leaves of OF2 sites, aluminium in roots of PS1 sites and zinc in the Amaranth leaves and roots of all PS sites (Fig. 1). Significant differences between metal concentrations in roots and leaves were observed only for Al and Fe in OF1 sites, for Cu in all OF sites, for Zn in OF1 sites, and for Mn in all studied conditions except those of sites OF1. The "cultivation time" parameter seemed to have no significant impact on metal concentration in plant in the case of culture under shelter whatever the metal. But for Al and Fe, concentrations were significantly higher in roots of OF1 than in those of OF2. Cu and Mn were more concentrated in leaves of OF2 sites than in those of OF1. There were no differences between OF1 and OF2 for Zn concentrations in plant. With regard to "cropping practice", this parameter seemed to impact metal concentrations in Amaranth, but depending again on metal species and part of plant. Indeed, Fe and Al concentrations tend to be higher in roots of OF sites than PS, Cu concentrations higher in leaves of OF sites, but Mn and Zn concentrations tended to be higher in Amaranth of PS sites. Cd and Pb in Amaranth had levels below the detection limits recommended by FAO/WHO (1984), 0.3 and 5 mg/kg respectively. This suggests that the consuming of Amaranth cultivated in Libreville presents no lead and cadmium contamination risk for human and animal health. Out of the five quantified metals, the lowest concentrations were found for Cu in all parts of the plant. Maiti and Jaiswal (2008) reported the same observations on another Amaranth species.

The levels of Al and Fe ranged from 138 to 360 mg/kg and from 176 to 495 mg/kg in leaves respectively, from 108 to 1150 mg/kg and from 159 to 1444 mg/kg in roots respectively. Contents of Al and Fe in plants varied greatly, depending on soil and plant factors (Kabata-Pendias, 2011). Al and Fe levels in roots of OF1 Amaranth were higher than the others. OF1 soils presented also higher values of the following soils parameters: organic matter (OM), cationic exchange capacity (CEC), Kjeldahl nitrogen (NTK), clay content (Ondo *et al.,* 2013b). Al ions generally translocate very slowly to the upper parts of plants. Most plants contain no more than 200 mg/kg of Al in dry weight (Mossor-Pietraszewska, 2001). About 76% of all leaves samples analyzed in our study presented concentrations of Al above this value. Ondo *et al.* (2013a) showed that such concentrations of Al in leafy vegetables, such as Amaranth and Roselle, led to a high target hazard quotient above the reference dose. It seemed that soil acidity was responsible of these high concentrations in plant. Furthermore, Amaranth leaves have been reported to contain high concentrations of silica and high concentrations of silica have been reported to correlate with aluminum toxicity in vegetables (Njenga *et al.,* 2007). The complex physiology of the Al toxicity in plants is also reflected in interactions with Fe and Mn.

Al phytotoxicity is one of the main causes for low productivity in acidic soils (Wang *et al.,* 2010). The Al toxicity in acid soils is frequently associated with increased levels of Fe and Mn. The Al excess in plants is known to induce Ca deficiency or reduce Ca transport. Cu concentration in plants was between 1.8 and 46.7 mg/kg. It was significantly higher in leaves compared to roots ($p<0.01$). Cu concentration was highest in the leaves of Amaranth of OF2 sites, where the soils showed the lowest concentration of Zn (Ondo *et al.,* 2013b). The copper concentration in Amaranth leaves of OF2 (Fig. 1) were also higher than those reported by other studies for Amaranth cultivated onto uncontaminated soils in Africa (Agbenin *et al.,* 2009). The levels of Mn ranged from 17 to 375 mg/kg in the leaves, while in roots they ranged from 20 to 99 mg/kg. Generally, Mn is known to be rapidly taken up and translocated within plants (Kabata-Pendias, 2011). Except on OF1 sites, Mn levels were effectively higher in leaves than in roots, higher in PS Amaranth than OF Amaranth. Parallel to that, sand content and zinc concentrations in PS soils were higher than OF soils (Ondo *et al.,* 2013b). Zinc concentrations ranged from 37 to 159 mg/kg in the leaves, while in roots they ranged from 25 to 118 mg/kg. The highest concentrations of Zn were found in leaves and roots of PS samples. The Zn content within the same species was highly variable and ranged from 0.2 to 84 mg/kg in fresh matter of several Amaranth species (Uusiku *et al.,* 2010). In many parts of the developing world, most Zn is provided by edible parts of plants. These plants have high concentration of phytic acid, which is a potent inhibitor of Zn absorption. Also, high concentrations of this metal in vegetables could help to its supplementation by vegetables for populations in many developing countries where Zn deficiency is widespread (Olivares *et al.,* 2004). In areas, where iron deficiency has been reported, nutritional zinc deficiency is also common. This occurs because iron and zinc have similar distributions in the food supply and some dietary components affect the absorption of both iron and zinc (Uusiku *et al.,* 2010). *Amaranthus cruentus* is a popular vegetable in Africa, Asia and many tropical regions (Law-Ogbomo and Ajayi, 2009). This plant has been found to grow on a wide range of soil conditions. It can be cultivated satisfactorily on relatively infertile soils. The cultural practices which develop high Zn accumulation in plants will help the supplementation of this metal for the living people in these regions.

Relationship between soil properties and metals concentrations in Amaranth: Metal uptake in plants can be influenced by physical and chemical soil properties (Oguz *et al.,* 2006; Kabata-Pendias, 2011). To determine the properties affecting metal accumulation in leaves and roots of Amaranth, a correlation matrix was tested between mobile fractions of metals in soils (EDTA-extractable) and metals accumulated by plant and results are shown in Table 1.

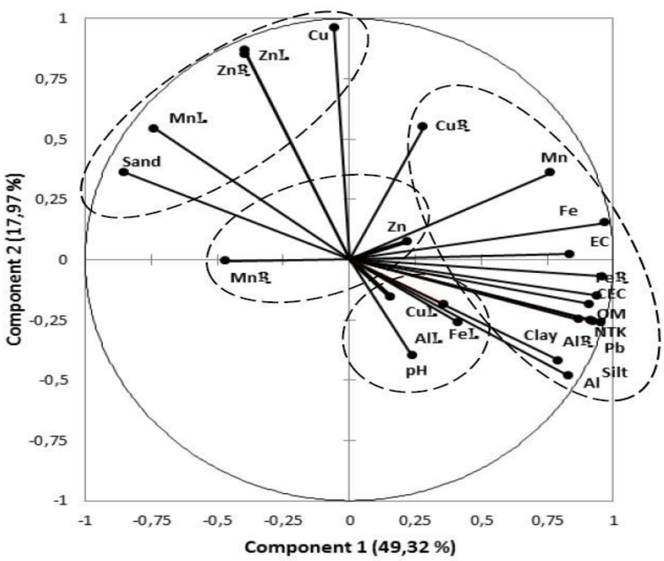
Table 1. Pearson correlation matrix between mobile fraction of metals in soils and metals in leaves and roots of Amaranth collected on the 11 studied sites.

	AlL	AlR	CuL	CuR	FeL	FeR	MnL	MnR	ZnL	ZnR
Al_m	-0.369	0.249	-0.128	-0.147	-0.341	0.010	-0.470*	0.096	-0.095	-0.140
Cu_m	0.437*	0.657**	-0.181	-0.043	0.592**	0.815***	-0.302	-0.053	-0.226	-0.202
Fe_m	-0.065	-0.359	-0.525*	-0.336	0.336	-0.151	0.717***	0.294	0.555**	0.531*
Mn_m	0.461*	0.791***	0.230	0.248	0.597**	0.930***	-0.484*	-0.538*	-0.384	-0.383
Zn_m	0.078	-0.482*	-0.634**	-0.482*	-0.146	-0.410	0.478*	0.946***	0.253	0.339
AlL	1									
AlR	0.348	1								
CuL	-0.193	0.138	1							
CuR	-0.175	0.093	0.466*	1						
FeL	0.582**	0.450*	-0.058	-0.427	1					
FeR	0.433*	0.942***	0.067	0.110	0.572**	1				
MnL	-0.062	-0.828***	-0.437*	-0.161	-0.073	-0.647**	1			
MnR	-0.060	-0.573**	-0.591**	-0.345	-0.347	-0.508*	0.515*	1		
ZnL	-0.272	-0.548**	-0.483*	0.243	-0.413	-0.442*	0.729***	0.370	1	
ZnR	-0.294	-0.608**	-0.420	0.291	-0.471*	-0.480*	0.745***	0.475*	0.972***	1

M_m: Soil metal mobilized by EDTA 0.05 M in soils, ML: Metal in Amaranth leaves; MR: Metal in Amaranth roots; *significant at $p<0.05$; **significant at $p<0.01$; ***significant at $p<0.001$.

Mobilizable metals in soil are considered to be easily absorbed by plants (Kabata-Pendias, 2011). We have used the standard method of Community Bureau of Reference (BCR) that allows extracting the mobile fraction of soil with a solution of 0.05 M EDTA (Quevauvillers, 1998). The first part of Table 2 presents several significant correlations between mobile metals in the soil and metals in leaves and roots of Amaranth. However, there is no significant correlation between the mobile fraction of one metal in soil and its concentration in Amaranth parts, except for Mn where these correlations were negatives (Table 1). The EDTA solution was not able to estimate the phyto-availability of metals in soils of Libreville region. A large data set from the literature by Menzies *et al.* (2007) suggests that generally, neutral salt extractants such as $CaCl_2$ and $NaNO_3$ provide better useful indication of metal phyto-availability than complex agents such as EDTA and DTPA. The second part of Table 1 presents correlations between metals in leaves and roots of Amaranth. Significant positive correlations were observed between Al and Fe in leaves and roots, and between Mn and Zn in leaves and roots. But significant negative correlations were also observed between Cu in leaves and Mn, Zn in leaves and roots and also between Al, Fe and Mn, Zn in leaves and roots. These results show interactions between metal uptake in leaves and in roots of Amaranth. The greatest number of antagonistic reactions has been observed for Fe, Mn, Cu and Zn, which are, obviously, the key elements in plant physiology. Bowell and Ansah (1994) in Ghana noted that Cu uptake was found to be antagonistic to Fe and Zn accumulation in Amaranth. Translocation of Mn and Zn from roots to upper parts of plants was inhibited with strong Al and Fe concentrations in roots. Celik *et al.* (2011) showed that high Al contents correlated with low Cu, Mn and Zn contents in roots and restrained Fe from being translocated into shoots and leaves.

Fig. 2. Principal component analysis of 14 soil parameters and 10 metal concentrations in plant of the 11 studied plots represented by components 1 and 2 as loads.



In order to better approach the interaction of soil characteristics on metal transfer to plant, a principal component analysis (PCA) was performed with physico-chemical properties and pseudo-total metal concentrations of soils and accumulated metals in Amaranth leaves and roots (Table 2 and Fig. 2). The first principal component (PC1) explained 49.3% of the total variance and had high positive loadings (>0.7) of most soil parameters (EC, OM, NTK, CEC, silt, clay, Al, Fe, Mn and Pb) and also of Al and Fe in Amaranth roots. The accumulation of these two metals in Amaranth roots seemed thus to be correlated to the level of soil parameters (as illustrated in Fig. 2).

Table 2. Rotated component loadings of 14 soil parameters and 10 metal concentrations in plant on significant principal components (PC), for the 11 studied plots.

Components	PC1	PC2	PC3	PC4
Variability (%)	49.32	17.97	12.68	11.26
% cumulated	49.32	67.29	79.96	91.22
CE	0.836	0.022	-0.123	0.469
pH	0.239	-0.399	-0.324	0.715
NTK	0.869	-0.249	0.229	0.230
OM	0.911	-0.185	0.253	-0.172
Sand	-0.855	0.364	-0.267	-0.193
Silt	0.793	-0.419	0.341	0.156
Clay	0.922	-0.254	0.132	0.225
CEC	0.937	-0.148	0.122	0.273
Al	0.833	-0.481	0.124	0.109
Cu	-0.058	0.962	-0.030	-0.196
Fe	0.971	0.153	-0.121	0.072
Mn	0.763	0.361	0.427	-0.159
Pb	0.952	-0.261	0.014	0.028
Zn	0.222	0.077	-0.869	-0.315
AlL	0.412	-0.260	0.353	0.440
CuL	0.156	-0.156	0.808	-0.243
FeL	0.356	-0.185	0.287	0.839
MnL	-0.738	0.545	-0.013	0.318
ZnL	-0.395	0.870	-0.151	-0.200
AlR	0.915	-0.251	0.078	0.034
CuR	0.282	0.551	0.314	-0.579
FeR	0.958	-0.070	0.083	0.214
MnR	-0.468	-0.006	-0.757	0.019
ZnR	-0.394	0.852	-0.221	-0.204

EC: electrical conductivity of soil; NTK: total Kjeldhal nitrogen in soil; OM: organic matter in soil; CEC: cation exchange capacity of soil; M: pseudo-total metal in soil; ML: metal in Amaranth leaves; MR: metal in Amaranth roots.

The contents of Al and Fe were actually the greatest in the roots of Amaranth cultivated on OF1 sites, where the characteristics of soils were in agreement with the last observation (i.e. high values of OM, CEC, Al, Fe, Mn). PC2 explains 18% of the total variance and had positive loadings (>0.5) of pseudo-total Cu in soils, Mn and Zn in leaves and of Cu and Zn in roots (see Fig. 2). PC3 explained 12.7% of the total variance and had positive loadings of Cu in leaves (>0.8) and had negative loadings of pseudo-total Zn in soil and Mn in roots of Amaranth. PC4 explained 11.3% of the total variance and had positive loadings of pH and Fe in leaves and had negative loadings of Cu in roots of Amaranth.

Bioconcentration factors of metals in Amaranth: To characterize the relationship between soil metal content and metal supply to the plant, we determined the metal transfer coefficient between soil and plant. The metal transfer coefficient is usually defined as plant metal content divided by soil pseudo-total metal content. Bioconcentration factor (BCF) is known as the uptake factor of metal from soil to plant (Tiwari *et al.,* 2011). The uptake factor of metal for leaves (BCF_{leaf}) and for roots (BCF_{root}) of Amaranth decreased in the order Zn > Cu > Mn > Al > Fe, respectively (Table 3). In general, BCF_{leaf} was found above BCF_{root} for Cu, Fe, Mn and Zn, with some exception.

In the case of Al, BCF_{root} was above BCF_{leaf} for Amaranth cultivated on PS2, OF1 and OF2 sites. Al, Mn and Zn were significantly more taken up in Amaranth of PS than Amaranth of OF sites, while Fe and Cu seemed to be more accumulated in Amaranth of OF than Amaranth of PS sites. BCF >1 were recorded for Cu in leaves of plants cultivated on OF2 sites, for Mn in leaves of plants cultivated on PS1 sites and for Zn in leaves and roots of all Amaranth whatever the cultural and duration practices, except in roots of OF1 (Table 3).

BCF was found lower for Al and Fe, metals of lithogenic source. High BCF values of Zn in edible parts of Amaranth confirms that this vegetable takes up and accumulates high Zn levels and could be recommended as food for the supplementation of Zn in the case of deficiency this metal. The results of this study were quite similar to the results reported in the literature suggesting that Amaranth species could be good accumulators of metals such as Cu, Mn and Zn (Tyokumbur and Okorie, 2011), in non-specific contaminated conditions. Availability of metal from root to leaves can be assessed by simple index termed as Translocation Factor (TF). TF can be defined as the ratio between the metal concentrations accumulated in leaves divided by the metal concentrations accumulated in roots.

Table 3. Means of bioconcentration factors in leaves (BCF_{leaf}) and in roots (BCF_{root}) and translocation factor (TF) of metals in Amaranth cultivated on the different studied sites.

Metals	Sites	PS1	PS2	OF1	OF2
Al	BCF_{leaf}	0.324a	0.030b	0.013b	0.015b
	BCF_{root}	0.158a	0.052b	0.040b	0.028b
	TF	2.039a	0.691b	0.321b	0.587b
Cu	BCF_{leaf}	0.230a	0.638a	0.563a	2.460b
	BCF_{root}	0.181a	0.369a	0.338a	0.688b
	TF	2.264ab	1.594b	1.622ab	3.283a
Fe	BCF_{leaf}	0.035a	0.017a	0.019a	0.031a
	BCF_{root}	0.015a	0.014a	0.036b	0.027c
	TF	2.489a	1.148bc	0.553b	1.299c
Mn	BCF_{leaf}	1.219a	0.660b	0.201c	0.268c
	BCF_{root}	0.199ab	0.346b	0.113a	0.069a
	TF	6.107a	2.280b	1.390b	4.845a
Zn	BCF_{leaf}	3.767a	1.933b	1.342b	1.997b
	BCF_{root}	2.720a	1.517b	0.720b	1.358c
	TF	1.385b	1.236b	1.828a	1.503ab

This ratio shows metal translocation properties from roots to leaves (Maiti and Jaiswal, 2008). The data indicate that Cu, Mn and Zn accumulated by Amaranth grown on all soils and that Fe accumulated by Amaranth on PS sites, were well translocated in the leaves, as shown by TF mean values >1 (Table 3). Al accumulated in Amaranth cultivated on all sites and Fe accumulated in Amaranth of OF cultivated on OF sites, were retained to a greater extent in the roots (TF <1). The rate and extent of the translocation within plants depended on the metal and agricultural systems concerned. For e.g., the translocation of Cu was more important in Amaranth cultivated on OF sites than in Amaranth on PS sites and the translocation of Al and Fe was more important in Amaranth on PS sites than in Amaranth on OF sites. Mn was the metal which was more translocated (TF >3) than the other metals. Generally, Mn is known to be rapidly taken up and translocated within plants. The Mn content of plants depends of plant characteristics and the pool of available Mn, which is highly controlled by soil properties like the pH. Generally, the most readily available Mn is in acid soils. It appears, therefore, that Mn is likely to be transported as Mn^{2+} and accumulated in leaves with age, particularly in a high Mn supply (Kabata-Pendias, 2011).

Conclusion

This study has shown that variation in bioaccumulation of studied metals (Al, Cu, Fe, Mn and Zn) in *Amaranthus cruentus* depend on many factors such as soil physic-chemical properties, pseudo-total metal content in soil, parts of plant concerned (roots or leaves), possible synergism or antagonism between metals, as well as cropping practice and time of cultivation. The use of 0.05 M EDTA for phyto-availability assessment of metals was not conclusive and suggests that other solutions should be used to determine bioavailable metal fractions in soils. The assessment of metal concentrations showed that *A. cruentus* exhibited high Al concentrations and sometimes high Zn concentrations than in other studies on Amaranth vegetables. Shelter promoted the bioconcentration of Al, Mn and Zn and translocation of Al, Fe, Mn and Zn by *A. cruentus*, particularly for short time of cultivation. Considering that toxic Al and essential Zn may be accumulated in vegetables and could pose potential threats to human health, systematic studies of metal behavior in cropping systems as influenced by different cultivations time, may be recommended to gather practical information on safe production of crops.

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