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Assessment Of Heavy Metals In Amaranthus Spinosus, Kigali, Rwanda.

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Abstract: Spiny amaranths "Amaranthus spinosus" is a common leafy vegetable and is locally called dodo. It is grown both in wet and dry seasons and harvested between 30-40 days after planting. Because of its nutritional importance and demand but also less expensive it is widely cultivated especially in Kigali, Rwanda. The level of heavy metals in soil and leaf samples of Amaranthus spinosus grown in seven sites were performed by using Inductively Coupled Plasma Emission. The objective of study was to assess the level of heavy metals in soil and in leaves of amaranthus spinosus. The heavy metals that were assessed are: Manganese (Mn); Zinc (Zn); Copper (Cu); Cadmium (Cd); Iron (Fe); Nickel (Ni) and Lead (Pb). The results obtained revealed that their concentrations in soil ranged between : Mn (45.3-243.5); Zn (37.8-56.7) ; Cu (5.3-44.6); Cd (0.09-0.21); Fe (1789-2896); Ni (9.6-23.8) and in the leaves the concentration of some heavy metals are ranged between: Mn (0.22-0.56); Zn (0.89-1.52); Cu (0.12-0.34); Fe (66.67-92,7); Ni (0.04-3.12) in mg/kg from all sites. The Lead (Pb) was not detected in soil and in leaves samples, and cadmium "Cd" is not detected in leaves samples. The values of all heavy metals analyzed from in whole samples in present sites were under the threshold values established by European organization and World Health Organization (WHO for food and vegetables). The results are an indication that the spiny amaranths leaves had the potential to be used as source of nutrients in alleviating macro- and micro- nutrient deficiencies. The study will bring awareness to consumers of these items about what they are taking and the health implication as well as assist them and the farmers in taking necessary precautions towards proper care of their fruits and vegetables before consumption. This will indeed be a good way of reducing substantial quantity of contaminants from getting to the human body thereby avoiding health problems, with its attendant positive economic implications.

Key words: Amaranthus Spinosus, heavy metal, soil, leave

1. Introduction

Spiny amaranth (**Amaranthus Spinosus**) as green leafy vegetables are inexpensive, are easily and quickly cooked, and are rich in several nutrients, such as vitamins, proteins, and minerals (Gupta K, Wagle1998) .It is a terrific source of minerals (manganese and also iron, calcium, copper, magnesium, phosphorus and potassium (Thompson and Kelly, 1990)). The originally Studies showed that optimal intake of elements, such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine, in amaranth could reduce individual risk factors, including those related to cardiovascular disease. Regular consumption of amaranth can help to reduce cholesterol levels and lower blood pressure (Gonor et al., 2006; Martirosyan et al., 2007). In the recent decades, the development of anthropogenic activities such as industry, agriculture and urban life has resulted in increasing of environmental pollution. According to the previous studies (Diaconu et al., 2009; Karbassi et al., 2014; F. X. Nshimiyimana and et al., 2016). Among the environmental pollutants, heavy metals represent a significant hazard to the environment, being both an ecological and health risk. The use of different chemical products such as fertilizers and pesticides in agriculture can be one of the sources for heavy metals in rural areas. These elements are highly persistent and not biodegradable contaminants, therefore, they accumulate in soil, then becoming accessible to plants or leaching to groundwater (Diaconu et al., 2009; Minkina et al., 2014; Smical et al., 2008).Therefore the heavy metals uptake by plants grown in polluted soils has been studied to a considerable extent (Wong et al., 1996 and Yusuf et al., 2003. However the human exposure to heavy metals has risen dramatically in the last 50 years, as a result of an exponential increase in the use of heavy metals in industrial processes and products(Marshall, 2004; Radwan and Salama, 2006; Wang et al., 2005; Khan et al., 2008). Even if vegetables are source of nutrients but the increase in human activities, especially with the application of modern technologies, pollution and contamination of these vegetables in their cultivated areas has become inevitable. The objective of this study was to evaluate the level of heavy metals such as Cadmium (Cd), Lead (Pb), Nickel (Ni), Copper (Cu), Iron(Fe), Manganese(Mn), and Zinc (Zn) in topsoil, and in leaves of spiny amaranths located in Kigali City mainly at Kabuye and Nyabugogo roads then assessing if their concentrations are not high to cause potential negative effect to human health.

2. MATERIALS AND METHODS

2.1. SAMPLING SITES DESCRIPTION

This study was conducted in seven sites located in Kigali City. Those sites are characterized by the combined effect of motor washing, car washing and vehicular emission from the buses roads. Vehicular emission from the bus express road which is washed by the rain and run into the soil, gasoline, heavy-duty oil and lubricating oil which are all supplemented with additives such as metallic soaps and organic metal compounds (e.g. Tetraethyl lead in gasoline) are a major contributor of heavy metals. These metals then found their ways into the environment when the oils are combusted or disposed of in an environmentally inappropriate ways (Ayres et al., 1994).

2.2. SAMPLE COLLECTION

The spiny amaranths leaves were obtained from cultivated farmlands located at located in Kigali City mainly Nyabugogo upstream (-1.88850^{0} latitude, 30.067580^{0} longitude) and Nyabugogo downstream (-1.942812^{0} latitude, 30.041623^{0} longitude). Each sample was used for comparative analysis of the leaves and soils from these different areas.

2.3. SAMPLE TREATMENT

Each sample of the vegetable leaf was washed thoroughly with water to remove soil and other particles on the leaf, effort was made to ensure that the washing was done in the manner is representative of local practices as possible and the samples were dried in oven. The dried samples were pulverized using pestle and mortar and kept in a labeled aluminum foil for prior to analysis. Then the samples were digested by using 2:1 ratio Nitric Perchloric acid. On cooling, the digest was filtered into a 50.0 ml volumetric flask and made up to the mark with distilled water.While, the Soil samples were oven dried at 60 °C for 24 h before being ground into a fine powder using a sterile mortar and pestle. The samples (2.5 g) were transferred into a crucible before being mixed with 10 mL of aqua regia, which consisted of HCl: HNO_3 (3:1). The mixture was the digested on a hot plate at 95 °C for 1 hour and was allowed to cool to room temperature. The sample was then diluted to 50 mL using deionized distilled water. The supernatant was filtered through Whatman N^{o}.42 filter paper and (<0.45 µm) Millipore filter paper.

2.4. HEAVY METALS ANALYSIS

The analysis of level of heavy metals in digested leaves and soil samples were analyzed using Inductively Coupled Plasma Emission (Schimadzu,ICPE9000). The values below detection limits of the equipment were coded with ND. The statistical analysis was performed using SPSS 20 software and for comparative analysis, the World Health Organization (WHO) thresholds were considered. The mapping of the study area and heavy metals distribution was performed using ArcGIS 10 software.

3. RESULTS AND DISCUSSION

This chapter concerns the results of laboratory analysis on heavy metals determination in spiny amaranths (Amaranthus spinosus) located in Kigali city from seven different sites.

TABLE 1: Results of heavy metals in soil samples

Locations	Mn	Zn	Cu	Pb	Cd	Fe	Ni
KS1 soil	176.2	52.6	44.6	ND	0.18	2387	12.3
KS2 soil	134.9	48.2	12.9	ND	0.15	2112	9.6
KS3 soil	243.5	55.9	5.3	ND	0.09	1798	18.5
KS4 soil	213.6	37.8	31.5	ND	0.16	1813	14.2
NS1 soil	92.8	56.7	28.7	ND	0.21	2896	11.9
NS2 soil	45.3	44.3	19.6	ND	0.12	2544	23.8
NS3 soil	76.1	49.7	12.4	ND	0.18	2736	15.4
Norm value (WHO,CEE) in mg/kg	2000	300	100	100	3	50000	50

KS soil : Kabuye samples; NS soil: Nyabugogo samples ; ND : not detected

The average concentration of heavy metals in soil samples collected at selected sites are presented in Tables 1. All the metals investigated were found to be present in the soil samples. Coefficient of variation values for most examined metals revealed no significant difference among sampling points. This might be due to the fact that the waste generated by the sawmill industries are almost the same in composition. The levels (mg/kg) of Mn, Zn, Cu, Pb, Cd, Fe, and Ni at various sampling points from all the sites ranged from 92.8 -243.5 ; 44.3 -56.7 ; 5.3 -44.6 ; 0.09 -0.21; 1798-2896 ; 9.6-23.8 respectively. With the exception of elevated levels of Fe at Nyabugogo down stream (1798- 2896 mg/kg) and Lead (Pb) which was not detected in all samples. The concentrations of the other metals were almost similar regardless the nature of the activities taking place in each sawmill, thus suggesting that the metals with similar trend were probably of natural origin with mild contribution from anthropogenic source. Metals such as Cd, Cu, Pb and Zn have been reported with high tendency to binding tenaciously to organic matter contained in the soil. Hence, the organic matter of soil is known to play a major role in determining the bioavailability of heavy metal (Lacatusu R., 2000;.EU.'2002). The consistently high load of iron recorded in all the sites (NS1 –NS3 and KS1) not surprising considering the fact that iron is one of the constituents (alloy) of the saws used in sawmill operations or wood processing. The wear and tears of the saws and other metal equipment used might have contributed to the concentration of this metal when compared with other metals in this research work. Moreover, it had been earlier stated by a good number of researchers that iron occurs in high proportion in Nigeria soil, implying that the concentration is contributed from both anthropogenic and crustal origin. The presence of iron in soils and plants is desirable (Eze S, Hilary M.,2008) since it is one of the metals that are essential to human biochemical processes, for example haemoglobin in the human blood system contains iron which aids blood formation (Okoye BCO., 1992).The Value of Zn (44.3-56.7mg/kg) obtained is lower than all other metals investigated except Fe and Mn but higher than 25.68± 4.67mg/kg reported for 10m for road side soil in Osogbo (Fakayode SO, Olu-Owolabi BI.,2003).The mean concentration of Zn is within the recommended limit in daily dietary allowance (RDA) ,1987).The magnitude of mean concentration of Pb, Zn, and Cu are in agreement with values obtained in the street dust of Istanbul (Yetimoglu EK, Ercan O., 2007). The concentration of copper in all the examined sites was moderately high when compared with some of the investigated metals (Jang YC, Townsend TG, Bitton G., 2002). The Cu contents in this study are higher compared for soil in Europe . The values of Cu (0.76-3.54mg/kg) obtained is similar to 2.44 and 4.21mg/kg reported for fertilizer blending companies by Haramai et al., 2004. In their study of heavy metal levels in industrial estate of Bauchi, Nigeria. The concentration of this metal might be connected to the deposition of the chemicals used in the wood preservation on the receiving soil. The value of Cd (0.09-0.21mg/kg) obtained could be compared with 0.45±0.12mg/kg reported for soil samples from high traffic area (Fakayode SO, Olu-Owolabi BI., 2003). However Cd was non-essential element of human health with high biological toxicity which mainly accumulates in surface soil. Its concentration should be monitored. The concentration of Ni content was low when compared with mean values reported by Peris et al., 2008. This implies that all the metals are moderately leached underground by probably contribution from soil erosion. This is supported by the fact that under anthropogenic enrichment the maximum content of pollution is observed in the surface layer of the soil (Vazhenin IG.1987). The concentration of Nickel is in ranges between 7.07 to 102 mg/kg which has obtained in UK (Environmental Agency., **2007**). However, the levels of all the metals were within the permissible limits for metals in soil/sediment. Compared with World Health Organization (WHO), Food and Agricultural Organization (FAO) and Standard Guidelines in Europe,The level of heavy metals in soil is low. The concentration of Mn and Pb assessed in soils for some heavy metals from irrigated farmlands irrigated with industrial effluents of nagaon, assam (India) were low except for Cd, Ni and which were high (Linton Hazarika ,et al ,2016). The level of the metals presents no significant exposure risk but, preventive measures are necessary to decrease the risk of heavy metal contamination of plants which are consumed by human beings and animals. Recommendations for agricultural policy on polluted lands are of great importance. Appropriate plants selection is one of the most promising methods of minimizing heavy metal transfer from soil to plants. In this case, non-edible crops and those relatively tolerant (cabbage, tomato) to the influence of heavy metals are preferred. In a moderately polluted land it is advisable to restrict farming to species which are more resistant to pollutants, where uptake of pollutants by plant would not produce any risk for consumers' health. Sea foods and fish have also been found to have high levels of cadmium, organic mercury and arsenic (Asaolu, S.S. ; 2003). For those eating significant amount of fish, the level can be monitored by direct food testing or stool test for current exposure levels. Air emissions control should be given priority in sawmills because of their contribution to a number of global and local environmental effects, such as global warming, acid rain, ozone layer depletion and photochemical smog. The level of heavy metals in the assessment were in range compared to heavy metals obtained in soil of Romania but low as; 0-1256.7mg/kg Mn ; 0-44 mg/kg Ni; 0-128.5 mg/kg Cu ; 0-96.11 mg /kg Pb and 0-275.35 mg/kg Zn. Then the level of heavy such as Cu,Pb ,Cd and Ni in soil were also in range of what found in Malaysia as 0.38-240.59 Cu; 0.01-0.68 and 0.69-2.40 mg/kg except for the Lead that has not been detected (ND). The concentrations of heavy metals in soil results were arranged in order Fe > Mn > Cu > Ni > Cd using mean, except for Pb that has not detected in all soil samples. The values of all metals determined were below the tolerable limits recommended by World Health Organization (WHO) and European Union (EU) except for Iron (1798- 2896 mg/kg) in soil samples from all sites respectively which were above the standard maxima of 0.01 mg/kg (W H O, 1993.). On the other hand, the concentration of lead was lower than EU upper limit of 300 mg/kg (European Commission EC, 1986) and was at lower concentration than the maximum tolerable levels proposed for agricultural soil, 90-300 mg/kg (A. Kabata-Pendias, A. Duka, 1991).

Table 2.Results of heavy metals in leaves

Locations	Mn	Zn	Cu	Pb	Cd	Fe	Ni
KS1 leaf	0.60	1.52	0.31	ND	ND	83.48	3.12
KS2 leaf	0.22	0.99	0.17	ND	ND	72.73	2.59
KS3 leaf	0.56	1.12	0.13	ND	ND	66.67	2.99
KS4 leaf	0.48	1.02	0.18	ND	ND	83.9	ND
NS1 leaf	0.33	0.89	0.12	ND	ND	68.7	ND
NS2 leaf	0.24	1.22	0.34	ND	ND	84.3	0.06
NS3 leaf	0.16	1.44	0.25	ND	ND	92.7	0.04
WHO/FAO Guidelines	500	60	2.5	0.50 -30	<2.4	400-500	0.02-50

KS leaf : Kabuye samples; NS leaf: Nyabugogo samples; ND : not detected

Table2, above shows the experimental values of heavy metals. Iron was the most abundant heavy metal detected in all samples especially in Nyabugogo downstream compared to Nyabugogo upstream , However the concentration of Lead and Cadmium were not detected means that they are present in very low concentration in all samples from these seven different sites. The presence of high concentration of some heavy metals in Nyabugogo samples is an indication of increased pollution due to various vehicle emission and other human activities like the application of fertilizers, pesticides, car washing and release of domestic wastes in spiny amaranths plantation. The concentration of Lead ,Zinc ,Cadmium and Nickel in this study were low compared to that have obtained in palm oil with 37.25 ,10.20,0.13 and 5.40 mg/kg respectively (Asemave K., Ubwa S.T., Anhwange B.A., Gbaamende A.G.,**2012**). Comparing with the results found in spinach and tomato analysis.The cadmium and lead concentration was low compared to one from Nyabugogo which were 0.31 and 0.03 mg/kg Pb and Cd respectively (Etale, A.2012); The Zn, Cd ,Fe , Cu , Mn and Pb content in the Amaranthus leaves of the studied area was low comparable to the values reported in African and Bangladesh Amaranthus leaves (Naser, H.M., Sultana, S., Mahmud, N.U., Gomes, R. and Noor, S., 2011). All the vegetables examined had the potential to take up all the analyzed heavy metals to their edible parts. However, the concentrations of heavy metals absorbed by these vegetables were within the FAO/WHO guidelines for metals in foods and vegetables (Chinyere GC, Madu FU, 2015). The concentration of Ni, Cd and Pb found in leaves samples were lower compared to Madina town findings except for Nickel which was between 1.51- 4.96 mg/kg . The concentration of Zn ,Cu ,Cd and Ni that were analyzed from Larrea tridentate in

University of Texas were high compared to my findings. The level of Cd, Pb and Mn found in vegetables in assessed of vegetables and soils for some heavy metals from irrigated farmlands irrigated with industrial effluents of nagaon, assam (India) were high and Ni was in range of my results. The concentration of Mn (21.5-68 mg/kg); Fe (185-215 mg/kg);Ni (4.5-11.9 mg/kg) ;Zn (40-82.7 mg/kg) ; Pb (0.9 -6.1 mg/kg) and Cu (1.3-16) obtained in Assessment of heavy metals uptake in leafy vegetables (amaranyhus) grown on long term wastewater irrigated soil in Bangalore,Karnata were high compared to the results obtained in the table 2 above, and also were in range of the concentrations of the heavy metals in the edible parts of the 22 species vegetables obtained in different areas in China were 0.004–2.361 mg/kg Pb, 0.002–2.918 mg/kg Cd, 0.155–3.125 mg/kg Cu, 1.151–54.65 mg/kg Zn, and 0.014–1.780 mg/kg As, respectively except for copper which was low and Cd and Pb that were not detected. Using average of the results for leaf analysis ,they are in order Fe > Ni > Zn > Mn > Cu ,except for Pb and Cd that were not detected.

4. RISK FACTOR ASSESSMENT

4.1. Transfer factor

Metal concentrations in the extracts of soils and plants were calculated on the basis of dry weight. The plant concentration factor (PCF) was calculated as follows:

$$PCF = \frac{\text{Concentration(plant)}}{\text{Concentration (soil)}}$$ (Cui, etal,2005)

4.2. Pollution load index (PLI)

The degree of soil pollution for each metal was measured using the pollution load index (PLI) technique depending on soil metal concentrations. The following modified equation was used to assess the PLI level in soils. WhereC soil (Samples) and C reference (Reference) represent the heavy metal concentrations as WHO normal values for soil.

$$PLI = \frac{\text{Csoil (samples)}}{\text{C references}}$$ (Liu etal, 2005)

4.3. Daily intake of metals (DIM)

The daily intake of metals (DIM) was determined by the following equation. Where, C metal, C factor,food intake and B average body weight of an adult represents the heavy metal concentrations in plants (mg kg-1), conversion factor, daily intake of vegetables and average body weight, respectively. The conversion factor 0.085 was used to convert fresh green vegetable weight to dry weight, as described by Rattan et al. (2005). The average daily vegetable intakes for adults and children were considered to be 0.345 and 0.232 kg person−1 day−1, respectively, while the average adult and child body weights were considered to be 55.9 and 32.7 kg, respectively, as used in previous studies (Ge, 1992; Wang et al., 2005).

$$DIM = \frac{\text{Cmetal}*\text{Cf}*\text{Dfood intake}}{\text{B average weight}}$$

4.4. Health risk index (HRI)

The health risk index (HRI) for the locals through the consumption of contaminated vegetables was assessed based on the food chain and the reference oral dose (RfD) by WHO/FAO standards for each metal.. The HRI <1 means the exposed population is assumed to be safe.

$$HRI = \frac{\text{DIM}}{\text{RfD}}$$ (US-EPA; 2002)

The data were statistically analyzed using a statistical package SPSS 22.0. The measures were expressed in terms of means of concentrations of each heavy metal at all sampling sites.

Table 1: Oral reference doses (RfDO) mg kg-1 day-1 for heavy metals by WHO/FAO standards

Heavy metals	FAO/WHO (Codex Alimentarious Commission,2013)
Fe	7.00E-01
Zn	3.00E-01
Cu	4.00E-02
Pb	4.00E-03
Cd	1.00E-03
Mn	1.4E-02
Ni	2.00E-02

Table 2: Results of PCF, PLI, DIM and HRI

					df	0.345		
					cf	0.085		
Heavy metal	Conc. reference	Mean conc. in soil	Mean conc. in leaf	PCF	PLI	DIM	RfD values	HRI
Mn	500	140.343	0.433	0.003	0.281	0.0002	0.014	0.0162
Zn	60	49.314	1.171	0.024	0.822	0.0006	0.3	0.0020
Cu	2.5	22.143	0.214	0.010	8.857	0.0001	0.04	0.0028
Pb	30	0.000	0.000	-	0.000	0.0000	0.0035	0.0000
Cd	2.4	0.156	0.000	0.000	0.065	0.0000	0.001	0.0000
Fe	500	2326.571	78.926	0.034	4.653	0.0414	0.7	0.0591
Ni	50	15.100	1.760	0.117	0.302	0.0009	0.02	0.0462

The pollution load index (PLI) of copper and iron is greater than 1 , means that the sampling sites are polluted by those heavy metals Cu and Fe , and others like Mn, Zn .Pb, Ni and Cd have PLI less than 1 ,means those sites are not polluted as established by USEPA,2013 standards, and As the HRI <1 for all sampling sites means the exposed population is assumed to be safe.

Keywords : Health Risk Index (HRI), transfer factor(PCF) , pollution load index(PLI),daily intake of metals (MID) ,conversion factor(Cf),oral reference dose (RfD),average daily vegetable intake for adults (df).

Conclusion and recommendations

Soil is a great geochemical reservoir for contaminant as well as a natural buffer for transportation of chemical materials and elements in the atmosphere, hydrosphere, and biomass. For this, it is the most important component of the human biosphere. As soil is an important constituent of the human biosphere, any harmful change to this segment of the environment seriously affects the overall quality of human life. The most adverse effect of heavy metals is that they can be introduced into the food chain and threaten human health. Agricultural products growing on soils with high metal concentrations are represented by metal accumulations at levels harmful to human and animal health as well as to the bio-environment.the concentration of lead and cadmium were below compared to what were observed in Colocasiaesculenta, Amaranthusspp. and Ipomoea batata from Nyabugogo marshland. In brief the result obtained for heavy metals do not show a significance difference to the results presented by Nigerian Institute of science and the values for heavy metals are within the range permitted by European Commission (EC,1986), and World Health Organization (WHO,2002). Also the health risk index (HRI) calculated in the table above using USEPA model is less than 1 means the consumption of these vegetables as food may not pose immediate danger to humans but prolonged consumption could lead to bioaccumulation and adverse health implication especially for Cd, As, Pb and Hg. In brief the result obtained for heavy metals do not show a significance difference to the results presented by Nigerian Institute of science and , the values for heavy metals are within the range permitted by European Commission (EC,1986), and World Health Organization (WHO,2002).Consumption of these vegetables as food may not pose immediate danger to humans but prolonged consumption could lead to bioaccumulation and adverse health implication especially for Cd, As, Pb and Hg. It is therefore recommended that those wastes that pose greater health hazards be properly recycled in order to reduce environmental pollution and/or soil degradation. Sorting of wastes at source and statutory regulations of wastes managements should be encouraged. Farmers should also be educated and encouraged not to cultivate in farmlands around dumpsites since such farms may be polluted by toxic heavy metals.

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