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Case Report

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DETERMINATION OF HEAVY METAL CONCENTRATIONS IN VEGETABLES IRRIGATED WITH MIXTURES OF WASTEWATER AND SEWAGE SLUDGE IN ARUSHA MUNICIPALITY: IMPLICATIONS FOR HUMAN HEALTH

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ABSTRACT

The present study was conducted to assess the heavy metals (Cu, Cd, Pb, and Cr) concentrations in wastewater used by vegetable small-scale farmers for irrigation. The concentration of heavy metal from soil and vegetables (Amaranths sp and Ipomoea sp) as a result of wastewater irrigation around Arusha Municipal wastewater stabilization pond were determined using Flame Atomic Absorption Spectrophotometer (FAAS). The results obtained from the edible parts of sampled vegetables (Amaranths sp and Ipomoea sp) in this study, showed concentrations of heavy metals (Cr, Cd, Pb, and Cu) was (1.40, 4.21), (309.87, 152.60), (0.50, 0.48), (281.69, 248.54)(mg /Kg) respectively. This result reflected the risk associated with exposure for the period of life expectancy considered, and the inhabitants are highly exposed to health risks associated to these metals in the order Cd was 225.121and 457.132, Cu was 9.17 and 10.39; Pb were 0.7081 and 0.7081 and Cr was 0.0014 and 0.0041, for Amaranths sp and Ipomoea sp respectively. The THQ in Cd and Cu is greater than one while other metals are less than 1 in all the vegetables species. Therefore, Cd and Cu pose a health risk to consumers, while other metals do not pose serious health risk concern. However, vegetable consumption was just one part of food consumption, the potential health risks for residents might actually be higher than in this study when other routes of heavy metals intake are considered.

Keywords: Heavy metals, health hazards, Hazardous Quotient, Lead, Oral Reference Dose

INTRODUCTION

Water scarcity is an important concern in Sub-Saharan Africa and even in some parts of the World which it may lead to a war crisis (Jaafarzadeh, 1996). Hence smallholder farmers opt to use municipal wastewater as an important alternative source of water for irrigation. On the other hands the high population growth, increased per capital water consumption and increased water requirements for irrigation and industrial activities, resulted in substantial decrease of utilizable water resources. Also increase in urban populations in developing countries residents search for better living standards, commercial, larger amounts of freshwater are diverted to domestic and industrial sectors, which generate greater volumes of wastewater (Asano *et al.,* 2007). There is growing public concern due to over the illegal cultivation of crops especially vegetables on soils amended with sewage sludge or irrigated with admixtures of sewage and sewage sludge. The scarcity of water has negative influence on human livelihoods, economic development, and environmental quality throughout the world. Small holder farmers are mainly interested in general benefits, like low cost water source, increased agriculture production, but they are of less concern on detrimental effects like heavy metal contamination of crops, soils, and problems related to human health issues. There is an epidemiological evidence for Ascaris lumbricoides infections for people who consume uncooked vegetables irrigated with wastewater (Kozan, *et al.,* 2005). The Helminth infections, especially hookworm and A. lumbricoides have higher importance in relation to occupation related risks compared to protozoan viral or bacterial, infections (Hassan, *et al.,* 2012). The most affected group is farm workers, due to the long duration of getting in touch with wastewater and polluted soils (WHO, 1996). Wastewater may contain different heavy metals depending upon the type of activities it is associated with. Therefore, long term use of this waste water, for irrigation of crops especially green leafy and other vegetables, may result in accumulation of heavy metals in the soil and their transfer to vegetables under cultivation (Singh *et al.,* 2004; Sharma *et al.,* 2007). These heavy metals may be absorbed by GLV and finally enter the food chain (Fytianos *et al.,* 2001). These Heavy metals are well known to be very harmful because of their non-biodegradable nature, long biological half-lives and their potential to accumulate in different body parts (Fytianos *et al.,* 2001). The intake of heavy metal can lead to altering of humans and animals healthiness state. Food safety issues and potential health risks make this as one of the most serious environmental concerns (Cui, *et al.,* 2004). Green Leafy vegetables (GLV) are important items consumed in many African homes. Apart from different varieties added to the menu, they are valuable sources of nutrients particularly in African rural areas where they contributes significantly to minerals, protein, fibers vitamins, and other nutrients which are usually in short supply in daily diets (Saria, 2016), It is valuable to note that consumption of abundant types of edible plants as sources of food could be beneficial to nutritionally marginal community especially in developing countries where poverty and climate is causing chaos to the rural populace (Nderumaki, *et al.,* 2017). In many Sub-Saharan countries due to rapid growing population the availability of minerals and vitamins in food is inadequate to meet the recommended standard. Minerals cannot be synthesized by animals they only source is either plants or mineral-rich water (Anjorin, *et al.,* 2010). This paper presents findings from contaminated green leafy vegetables irrigated by wastewater from Arusha municipal pond. The assessment was done to determine whether the concentration of heavy metal in the mass consumed vegetables is of alarming level and of concern to public health. This will encourage future studies as heavy metal contamination in GLV is a challenge in Tanzania if not well addressed.

MATERIALS AND METHODS

Sampling site: Samples were taken from oxidation pond area of Arusha municipality (Fig 1). Around this oxidation pond, horticultural activities are taking place where smallholder farmers are using waste water from oxidation pond to irrigate the vegetables.

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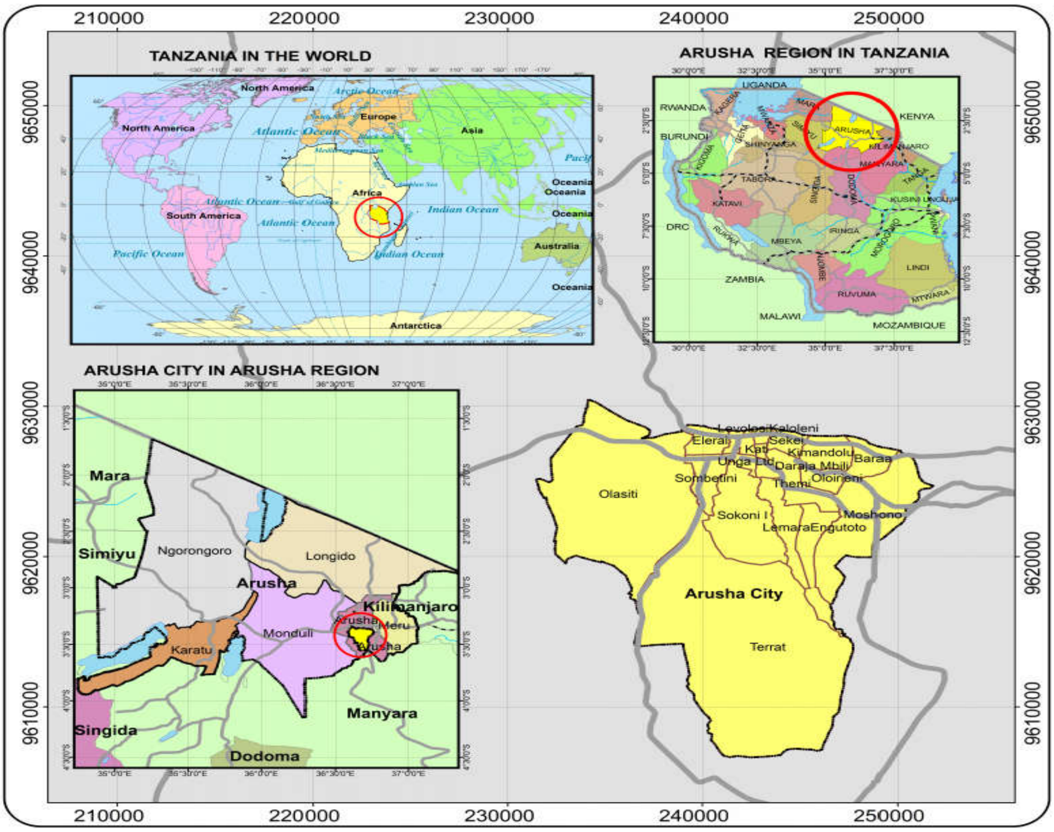

Fig 1 The Map of Arusha Municipality Showing the Sampling Site

Several industries like breweries, textiles, metals processing, electronic goods, fertilizers, pharmaceuticals, dyeing and battery manufacturing, discharge the effluents into the pond.

Soil Sampling and Treatment: Soil samples were taken at different depths at 5 cm intervals to a depth of 30 cm. Samples were collected into polyethylene bags, labeled and properly tied. In the laboratory, the soil samples were spread on glass plates and then dried in an oven at 105°C for six hours. The dried soil was ground and sieved through 0 –5 cm mesh sieve. One gram each of the ground soil samples was weighed into a 125 ml beaker and digested with a mixture of 4 ml, 25 ml and 2 ml each of concentrated $HClO_4$, HNO_3 and H_2SO_4 respectively, on a hot plate in a fume cupboard. On completion of digestion, the samples were cooled and 50 ml of de – ionized distilled water was added and then the samples were filtered. The samples were made up to 100 ml with de–ionized distilled water and concentrations of the elements determined using atomic absorption spectrophotometer (AAS Model SP 9 Unicam 1984).

Plant Sample and Treatment: The sampling and cleaning of the vegetable samples was done using a method previously procedure (fytianos *et al.,* 2001) .The samples were reduced to fine powder with a grinder prior to drying at 60oC in an oven to a constant weight. Half gram each of the fine powdered samples was weighed into a flask and digested in a mixture of 4 ml, 25 ml, 2 ml and 1 ml of concentrated $HClO_4$, HNO_3, H_2SO_4 and 60 % H_2O_2, respectively, at 100°C on a hot plate for two hours in a fume cupboard. The resulting solution was left over night and made up to 100 ml with de – ionized distilled water and concentrations of the elements determined using AAS.

Water Samples and Treatment: One liter of the grey waste water used for irrigating each farm was collected and treated with 1.5 ml of concentrated HNO_3. About 50 ml of the water sample was transferred to an evaporating dish and evaporated on a steam bath to about 20 ml.

Then 10 ml of 8 M HNO_3 of 98 % purity was added and evaporated on a hot plate to near dryness. The residue was quantitatively transferred using two aliquot of 10 ml and 15 ml of concentrated HNO_3 into a 250 ml flask. 20 ml of $HClO_4$ was added and boiled until the solution became clear and white fumes of $HClO_4$ appeared. It was then cooled and de – ionized distilled water (about 50 ml) was added and the solution filtered. The filtrate was quantitatively transferred to a 100 ml volumetric flask with two portions of 5 ml of de –ionized distilled water. The solution was diluted to mark and mixed thoroughly by shaking.

Soil-Vegetable Transfer Coefficients

The transfer coefficient quantifies the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties. The coefficient is calculated by dividing the concentration of a metal in a vegetable crop (DW) by the total metal concentration in the soil. Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong sorption of metals to the soil colloids (Coutate 1992). Soil to plant transfer is one of the key components of human exposure to metals through food chain. Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe not only the transfer of trace elements from soil to plant body but also function of both soil and vegetables properties. The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil (Kachenko and Singh 2006).

$$TF\,(PCF) = \frac{Conc_{\,(Vegetable)}}{Conc_{\,(Soil)}} \quad .... \quad (1)$$

Daily Intake Rate (DIR): The Daily Intake Rate (DIR) is the average metal content in each vegetable was calculated and multiplied by the respective consumption rate. The DIR was determined by the following equation (Sajjad *et al.,* 2009; Arora *et al.,* 2008):

$$DIR = C\,(\text{Metal conc. in Vegetable}) \times C\,(\text{Factor}) \times D\,(\text{Vegetable intake}) \qquad (2)$$

Where, C (Metal conc.) = Heavy metal concentration in vegetables (mg/kg); C (Factor) = Conversion factor (0.085); D (Vegetable intake) = Daily Intake of Vegetable (kg person^{-1}day^{-1} FW). The conversion factor of 0.085 is set to convert fresh vegetable weight to dry weight based on calculation in literatures (Rattan et al. 2005; USDA 2007). For daily vegetable consumption was obtained through a formal survey conducted in the Dar es salaam City. An interview of 60 persons of 35-65 years age group and of 50-77 kg body weight was conducted about their daily consumption rate of vegetables tested. An average consumption rate of each vegetable per person per day was calculated. The average daily intake for Tanzanian adults was set to be 0.246 Kg person^{-1}day^{-1} (expressed as fresh weight). According to WHO (1989) guidelines, the required amount of vegetables in our daily diet must be 0.300 to 0.350 Kg per person.

Hazardous Quotient (HQ): Hazardous Quotient (HQ) for the locals (consumers) through the consumption of contaminated vegetables was assessed by the ratio of Daily Intake Rate (DIR) to the oral reference dose (RfDo) for each metal (USEPA, 2013) where for Pb and Zn is 0.0035 and 0.300 mg/kg/day respectively (USEPA 2010). The target hazard quotient (THQ) was calculated using equation (3):

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA} \times 10^{-3} \qquad (3)$$

Where EF is the exposure frequency (350 days/year); ED is the exposure duration (According to the latest WHO data published in 2015 life expectancy in Tanzania is 62 years; lifetime); FIR is the food ingestion rate. According to Weinberger and Swai (2006) vegetable consumption values for Northern adult Tanzanian is 63 g/person/day); C is the metal concentration in the edible parts of vegetables (mg/kg); RFD is the oral reference dose (Pb = 0.0035 while Zn = 0.300), WAB is the average body weight (65 kg for adults vegetable consumer in Tanzania) and TA is the average exposure time for non-carcinogens (ED x 365days/year). If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects.

RESULTS AND DISCUSSION

Heavy metal concentrations in soil, water and vegetables: The average levels of heavy metals in the soil, water and vegetables are summarized in Table 1. Heavy metal pollution, which is caused by anthopogenic activities, is one of the serious ecotoxicological problems these days. There are different reports indicated that when wastewater is used for the irrigation of edible plants for long time, soil health is affected (Barman *et al.,* 2000; Singh *et al., 2004*).

Table 1. Heavy Metal Concentration in Different Sample (mg/kg)

Metal	Soil (mg/kg dw)	Wastewater (mg/L)	*Amaranthus sp* mg/kg dw	*Ipomoea sp* mg/kg dw
Copper (Cu)	234.52	237.82	281.69	248.54
Chromium (Cr)	0.65	0.32	1.40	4.21
Cadmium (Cd)	595.90	607.61	309.87	152.60
Lead (Pb)	48.00	0.15	0.50	0.48

The concentration of Cd in soil is higher than Maximum Allowable Limits (MAL) for heavy metals in soil used in different countries, where the recommended concentration in Germany is 500 mg/kg (Weichang, *et al.,* 2012). The level of Cd in wastewater 607.61 mg/L which is extremely high than the one obtained earlier 5.059 mg/kg by Chaoua *et al.,* (2019). The mean level in green leafy vegetables detected high in *maranthus sp* 309.87 mg/kg dw and lowest in ipomoea sp 152.60 mg/kg dw. These values are 300 times higher than range of cadmium obtained in waste water irrigated leafy vegetables determined earlier in Spinacia oleracea (1.05 mg/kg) (Khan, *et al.,* 2019). Cadmium is very soluble and easily leached by rain- water or swept by rainwater especially when the soil pH is in the acidic range. It is believed that the greater level of cadmium is bounded in the organic matter of the soil sample, therefore probably indicates that the greater tendency for cadmium to become unavailable once it is in soils without organic matter. The association of cadmium with organic soil may be due to high formation of organic-complexes chiefly from agrochemicals (Raj, *et al.,* 2006). The concentration of Cu in soil is 234.52 mg/kg, dw which is almost twice higher than the maximum acceptable limit by WHO (2012), which is 100 mg/kg, dw. However, the concentration was far less than the level detected 2,760 mg/kg dw, by Mwalaboko, *et al.,* (2014) at UDSM oxidation pond.

The average concentration of Cu in waste water is 237.82 mg/L. This value was approximately equal to the values obtained earlier by Ahmad (2010) which ranges between 10.7 201.29 mg/kg. In green leafy vegetables the range of concentration was 281.69 mg/kg d.w (*amaranthus sp*) to 248.54 mg/kg d.w (*ipomoea sp*). These values were about 18 times higher than the values determined earlier (Chove, *et al.,* 2006) which ranges between 8.85 to 13.9 mg/kg d.w. The high concentration of this Copper in green leafy vegetables functions as stress factors (Sarma, 2011). Soil contamination by copper is mainly attributed to agriculture activities such as continuous application of copper-based fungicides and pesticides application as fungicidal and bactericidal properties. Farmers have been employing copper compounds as a disinfectant on farms against storage rots and for the control and prevention of certain animal diseases, such as foot rot of animals. For example of such pesticides are copper-containing fungicidal sprays such as Bordeaux mixture (copper sulphate) and copper oxychloride (Jones, and and Jarvis, 1981). The concentration of lead in the samples were 4.00 mg/kg d.w in soil, 0.15 mg/L of waste water used for irrigation while levels in green leafy vegetables are 0.50 mg/kg d.w in *amaranthus* and 0.48 gm/kg d.w in *Ipomoea sp*. The levels in green leafy vegetables correspond with those detected earlier by Bahemuka and Mubofu, (1999), as well as those detected by which ranges between 0.19 – 0.66 mg/kg but higher that those detected by Mwegoha and Kihampa (2010) which ranges between 0.113 to 0.083mg/kg respectively.

Heavy metal transfer coefficient from soil to vegetables: Soil to plant transfer is one of the key components of human exposure to metals through food chain. Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe not only the transfer of trace elements from soil to plant body but also function of both soil and vegetables properties. The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil (Kachenko and Singh 2006). The bioavailability of heavy metals in soil is assumed to be available to all plants. As a result, heavy metals can be transfered to plant when they are in a mobile form. To estimate the heavy metals transferred to plants, the transfer coefficient (equation 1), a function of both soil and plant properties, is used due to its representative bioavailability of heavy metals to plants. Figure 2, higher transfer coefficient show the order of Cr > Cu > Cd >Pb in both *Amaranths sp* and *Ipomoea sp* leaf, which represents relatively poor retention of Chromium in soils or greater efficiency of plants to absorb, while the least heavy metals to be absorbed by plants is Pb, TF = 0.01 in both plant species. Low coefficient demonstrates the strong sorption of metals to the soil colloids.

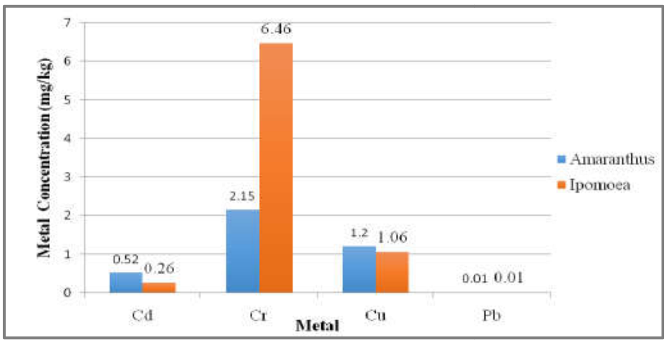

Fig. 2. Heavy Metal Transfer Coefficient from Soil to Vegetables

These results agrees with the study by Satter (2012) who found that the transfer of Zn and Pb from soil to plant *Enhydrafluctuans* and *Oryza sativa* is 1.762 and 1.05; and 5.519 and 1.20 respectively.Soil electrolyte plays an important role in the process of metal transfer. Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong sorption of metals to the soil colloids (Coutate 1992). The electrochemical properties of soil reflected through the temperature, pH, and electrolyte concentration etc. thus influence the migration transformation ability of toxic metal indirectly (Iyaka *et al.,* 2014).

Daily intake rate of heavy metals: The Daily Intake Rate (DIR) is the average metal content in each vegetable was calculated and multiplied by the respective consumption rate. The DIR was determined by the following equation (Sajjad *et al.,* 2009; Arora *et al.,* 2008):

$$DIR = C_{(\text{metal conc in Vegetable})} \times C_{(\text{Factor})} \times D_{(\text{vegetable intake})} \quad \text{.........................(1)}$$

Where, C (Metal conc.) = Heavy metal concentration in vegetables (mg/kg); C (Factor) = Conversion factor (0.085); D (Vegetable intake) = Daily Intake of Vegetable (kg person^{-1}day^{-1}. The conversion factor of 0.085 is set to convert fresh vegetable weight to dry weight based on calculation in literatures (Rattan *et al.,* 2005; USDA 2007).

For daily vegetable consumption was obtained through a formal survey conducted in the Dar es Salaam City. An interview of 60 persons of 35-65 years age group and of 50-77 kg body weight was conducted about their daily consumption rate of vegetables tested. An average consumption rate of each vegetable per person per day was calculated. The average daily intake for Tanzanian adults was set to be 0.246 Kg person^{-1}day^{-1} (expressed as fresh weight). According to WHO (1989) guidelines, the required amount of vegetables in our daily diet must be 0.300 to 0.350 Kg per person. The daily intake of metals (DIM) results in Table 2 was compared with the recommended daily intake of metals and the upper tolerable daily intake level (UL) for people between the ages of 19 to 70 years (FDA, 2001; Garcia-Rico, 2007).

Table 2. Daily Intake Rate (mg per person per day) of Heavy Metals

Vegetables	Cu	Cr	Cd	Pb
Amaranths sp	36.84	0.18	40.52	0.065
Ipomoea sp	32.50	0.55	19.96	0.063
DIM (mg per day per person)	0.90	-	0.00	0.00
UL (mg per day per person)	10.00	2.47	0.064	0.240

Recommended daily intake (DI) and upper tolerable daily intake (UL) levels of heavy metals in foodstuffs (FDA, 2001; Garcia-Rico, 2007)

Results show that daily intake of metals in vegetables species for Cr (0.18 – 0.55 mg/kg/person) and Pb (0.065– 0.063 mg/kg/person) are significantly higher than the recommended daily intake of metals and lower than the upper tolerable daily intake level (UL). However, DIM of Cd (40.52 – 19.96 mg/kg) and Cu (36.84 – 32.50 mg/kg) exceed the recommended DIM and the upper tolerable daily level. The DIM of Cr (0.048 – 0.082 mg/kg/person) is lower than the recommended oral reference dose (RfD) of 1.5 mg/kg (USEPA, 2010).

Human Health Risk Index (HHIR) and Hazardous Quotient (HQ): Human Health Risk Index (HHIR) for the locals (consumers) through the consumption of contaminated vegetables was assessed by the ratio of Daily Intake Rate (DIR) to the oral reference dose (RfDo) for each metal (Oral reference dose (USEPA IRIS, 2006).

The Human Health Risk Index (HHIR) was calculated using equation (eq 3):

$$HHRI = DIMRFD \quad \text{..(2)}$$

DIM = The Daily Intake of Metals
RFD = Oral Reference Dose

The target hazard quotient (THQ) was calculated using equation (3):

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA} \times 10^{-3} \quad \text{..............................(3)}$$

Where EF is the exposure frequency (350 days/year); ED is the exposure duration (According to WHO data published in 2018 life expectancy in Tanzania is: Male 62.0, female 65.8 and total life expectancy is 63.9 (WHO, 2018); FIR is the food ingestion rate. According to Weinberger and Swai (2006) vegetable consumption values for Northern adult Tanzanian is 63 g/person/day); C is the metal concentration in the edible parts of vegetables (mg/kg). The WAB is the average body weight (65 kg for adult's vegetable consumer in Tanzania) and TA is the average exposure time for non-carcinogens (ED x 365days/year). If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects.

Table 3. Results of Calculated HHRI and HQ for all the Vegetables Species

Vegetables	Cu		Cr		Cd		Pb	
	HHRI	THQ	HHRI	THQ	HHRI	THQ	HHRI	THQ
Amaranths sp	921	405.79	0.12	0.053	40520	17855.56	18.57	8.23
Ipomoea sp	812.5	358.04	0.37	0.16	19960	8793.23	18	7.91
Oral reference dose (RFD)	0.040		1.500		0.001		0.0035	

The HHRI was; Cu (921 and 812.5), Cr (0.12 and 0.37), Cd (40,520 and 19,960), and Pb (18.57 and 18) for *Amaranths sp* and *Ipomoea sp* respectively. The HHRI for Cu, Cd and Pb from this study were far greater than 1 (HHRI > 1) except chromium. Generally, HHRI < 1 means that the exposed population is safe of metals health risk while HHRI > 1 means there is potential health risks (Khan *et al.,* 2008). The population is therefore at greater risk of Cu, Cd, and Pb. The THQ is a ratio between the measured concentration and the oral reference dose, weighted by the length and frequency of exposure; amount ingested and body weight (NRC, 1983). The parameter defines the exposure duration and the risk with that period. The THQ values of Cu, Cr, Cd, and Pb due to vegetable consumption for the population (adults) of the study area are listed in Table 4.5. The THQ values showed that Cd was 17855.56 and 8793.23, Pb were 8.23 and 7.91, Cr was 0.053 and 0.16, and Cu was 405.79 and 358.04 for *Amaranths sp* and *Ipomoea sp* respectively. This result reflected the risk associated with Cd, Pb, Cr, and Cu exposure for the period of life expectancy considered, and the inhabitants are highly exposed to health risks associated to these metals in the order Cd > Cu >Pb> Cr. Generally, Cu which is important nutrients for humans is considered a much lower health risk to humans than Pb, and Cd (Brewer, 2001). In this study, the THQ in Pb and Cr metals is far less than 1 in all the vegetables species; therefore, it does not pose health risk concern. The Cd and Cu have THQ higher than 1 in all vegetables species, therefore pose health risk concern. Higher THQ for Cd, Pb, and Ni were also reported earlier by Singh *et al.,* (2010) in vegetables from wastewater irrigated area. The potential health risks of heavy metal accumulation through vegetable consumption were likely to be higher than for the normal population. However, vegetable consumption was just one part of food consumption. For Arusha population, food consumption, air pollution, drinking water is the important pathways for human exposure to toxic metals. Consequently, the potential health risks for the residents were actually higher.

Conclusion

Among analyzed vegetable tested: *amaranths sp* has a high metal content than *ipomoea sp,* which makes the daily intake (mg/person/day) to be in the order of Cu (36.84) > Cd (40.52) > Cr (0.18)> Pb (0.065). The potential health risks of heavy metal accumulation through vegetable consumption were likely to be higher than for the normal population. However, vegetable consumption was just one part of food consumption others important pathways for human exposure to toxic metals may be air pollution, drinking water and fruit consumption. Consequently, the potential health risks for the residents were actually higher.

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