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Investigation of possible human exposure to metals concentration in vegetables

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This study assesses the health risk of metals concentration in spinach and onions obtained from different suppliers (suppliers 1, 2, 3, 4 and 5) in Mafikeng, South Africa. The following elemental concentrations of As, Ba, Bi, Cu, Cr, Co, I, Mn, Ni, Pb, Sr, Th, Rb, V and U were tested in spinach and onions using inductively coupled plasma–mass spectrometry (ICP-MS). The result shows that Bi, I, Th, Pb and U were not detected in all the samples of spinach and onions. In spinach, the degree of decreasing abundance is as follows: Mn > Cr > Sr > V > Rb > As > Ba > Cu > Ni > Co, while in onions it is Sr > Mn > V > As > Cr > Cu > Ba > Rb > Ni > Co. The estimated daily intake of metal (DIM) for As and Cr in spinach from suppliers 1, 2, 3, 4 and 5 were higher above the limit values of 0.020 and 0.033 mg/kg recommended by Food and Agriculture Organization (FAO)/World Health Organization (WHO), while, Ba, Cu, Co, Mn, Sr and V were below the limit values. The estimated DIM for As in onions was higher than 0.020 mg/kg recommended by FAO/WHO, while the intake for Ba, Cr, Cu, Mn, Ni, Sr and V were below 0.033, 2-3, 2-5, 01 and 10 mg/kg recommendations by FAO/WHO, respectively. The target hazard quotient (THQ) value in spinach obtained from suppliers 1, 2, 3 and 4 were less than 1, while, the THQ value for Cr is 2.19 greater than 1 in spinach obtained from supplier 5. The THQ value was less than 1 in onions obtained from all the suppliers. Spinach were not suitable for human consumption due to estimated DIM for As and Cr that were higher than the recommendation of FAO/WHO and THQ value of Cr that was greater than 1.

Key words: Spinach, onion, metal, estimated daily intake of metal, target hazard quotient.

INTRODUCTION

Human exposure to metal compounds in the general environment is usually greater through food and drink than through air (Nordberg et al., 2015). Metals and their

compounds occur naturally in food and drinking water, because they are intrinsic components of the earth's crust. Depending on geological variation and

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agricultural and ecological processes, there are great geographical differences in metal intakes among populations living in various parts of the world (Nordberg et al., 2007). Metal can also originate from pesticide, herbicide and fungicide (Gilbert, 2004), used to protect the vegetables from pest, herbs and fungi during the production stage.

Air, soil and water pollution contribute to the presence of harmful elements, such as Cd, Pb, and Hg in foodstuff; however, accumulation of harmful trace elements in vegetables has become an unavoidable challenge. The accumulation of heavy metal in an environment originates from rapid industrial growth, increased use of chemicals in the agricultural sector, and other urban activities (Orisakwe et al., 2012). These agents have led to metal dispersion in the environment and, consequently, impaired health of the population (Zukowska and Biziuk, 2008). During rainy season, water runoff cause leaching of harmful trace elements from dump sites to farm area that lead to metal uptake by root of crops (Orisakwe et al., 2012). Some metals are essential elements, and their deficiency results in impairment of biological functions. However, when present in excess, the elements may be toxic (Gunnar et al., 2005). Heavy metals can be very harmful to the human body even in low concentrations as there is no effective excretion mechanism (Ghosh et al., 2012) to reduce the effect.

A human health risk assessment is done to determine the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media (USEPA, 2015). These methods are typically based on estimated daily intake of metal (DIM) and the target hazard quotients (THQ). The contamination of foods by heavy metals has currently become a challenge (Orisakwe et al., 2012). It is therefore necessary to assess daily intake through the consumption of contaminant. THQ was developed by the United States Environmental Protection Agency (USEPA) for the estimation of potential health risks associated with long term exposure to chemical pollutants (USEPA, 2000). This method of risk estimation has recently been used by many researchers (Wang et al., 2005) and has been shown to be valid and useful.

Health risk associated with heavy metals accumulation in vegetables is still limited in Southern Africa. This study evaluated the potential health risks associated with heavy metals consumption through spinach and onions by using Metal concentration in vegetables, daily intake of metal (DIM) and THQ.

MATERIALS AND METHODS

Vegetables sample collection

The spinach and onions were obtained from three vegetable

suppliers herein identified as suppliers 1, 2, 3, 4 and 5 in Mafikeng, South Africa. It is situated in Molopo, North West, South Africa, its geographical coordinates are 25° 52' 0" South, 25° 39' 0" (Figure 1).

The vegetables samples were tightly sealed in plastic bags labeled appropriately and transported in a cooler box to the laboratory where drying commenced immediately. Samples were cut in small pieces, placed on aluminum plates and inserted in an oven overnight (12 h) at 60°C. The samples were grounded into fine powder with an electric grinder. One gram samples were collected from each sample and ashed in a furnace at 600°C for 2 h.

Ashed samples were digested in a solution of 8 ml nitric acid (55%) and 2 ml hydrochloric acid (33%). The digested solution was heated in a Microwave Reaction System (Multiwave 3000) at 160°C for 35 min. After cooling, volume was adjusted to 100 ml with de-ionised water. Each sample was filtered (Whatman no. 1) and 10 ml of the digestates were analysed for all elements by inductively coupled plasma mass spectrometry (ICP-MS), Perkin-Elmer, Model NexION™ 300Q at Faculty of Agriculture, Science and Technology, North West University, Mafikeng, South Africa. Metal concentrations were calculated from the mean of three replicates and three spectrometer readings each and reported in mg/l.

Mean comparison of metal concentration

The data were analyzed using a Statistical Package for the Social Sciences (SPSS). Each measurement was repeated 3 times and ANOVA method was used to determine the difference in mean and standard deviation of metal contents in vegetable samples, the mean difference is significant at 0.05 levels. Mean for spinach and onions from different shops: 1, 2, 3 and gardens: 1 and 2 in homogeneous subsets were displayed using harmonic mean method with sample size = 3 and subset for alpha = 0.05.

Estimate of potential human exposure to heavy metals in vegetables

The health risks associated with the consumption of heavy metal concentration in vegetables were assessed based on the DIM and THQ of heavy metals. The calculations were based on standard assumption for US-EPA risk analysis (USEPA, 2015). The estimated daily intake of metals for adults was determined by the following equation:

$$DIM = \frac{C_{metal} (mg/g) \times \text{Daily Intake} (g/person/day)}{\text{Average Body Weight}}$$

Where: DIM is estimated daily intake of metal.

C_{metal} is the metal concentrations in vegetable (mg/g), estimated daily intake of metals (DIM) of metal concentration in spinach and onions such as As, Ba, Bi, Cu, Cr, Co, I, Mn, Ni, Pb, Sr, Th, Rb, V and U were performed. The evaluation of average daily intake of vegetable was 400 g/person/day and the adult average body weight used was 70 kg (EA-RSA, 2010).

Non-carcinogenic health effect

Target hazard quotient (THQ)

The health risks from consumption of vegetables by local inhabitants were assessed based on the THQ. THQ is the ratio between exposure and reference oral dose (RfD), used to express the risk of non-carcinogenic effects. If the ratio is equal to or greater than 1, an exposed population experiences health

Table 1. Oral reference dose values taken from regional screening level (RSL) under toxicity and chemical specific information (USEPA, 2016).

Metals	RfD is the oral reference dose values (mg/kg/day)
As	3.00×10^{-04}
Ba	2.00×10^{-01}
Cr	3.00×10^{-03}
Cu	4.00×10^{-02}
Co	3.00×10^{-04}
Mn	1.40×10^{-01}
Ni	2.00×10^{-02}
Sr	6.00×10^{-01}
V	5.00×10^{-03}

risks. The method of estimating risk using THQ was based on the following equation by USEPA (2016), based on the equation below:

$$THQ = \frac{MC \times IR \times EF \times ED}{RfD \times ABW \times AT}$$

Where: THQ is target hazard quotient, MC is metal concentration in vegetables, IR is ingestion rate mg/kg dry weight, EF is exposure frequency day/year (365) and ED is exposure duration for 30 years. RfD is the oral reference dose values were taken from regional screening level (RSL) under toxicity and chemical specific information (USEPA, 2016) as shown in a Table 1. ABW is average body weight for an adult used was 70 kg and AT is average exposure time for non-carcinogenic effects (365 days per year over 30 years (10950 days) (EA-RSA, 2010). The THQ has been recognized as a useful parameter for evaluating risk associated with consumption of metals contaminated vegetable.

RESULTS AND DISCUSSION

Metal concentrations

The result shows that Bi, I, Th, Pb and U were not detected in all samples of spinach. There was no significant different among all metal tested in spinach samples from suppliers 1, 2, 3, 4 and 5. The metal concentration in spinach were in an ascending order of Mn > Cr > Sr > V > Rb > As > Ba > Cu > Ni > Co as shown in Table 2.

The result shows that Bi, Co, I, Th, Pb and U were not detected in all samples of onions. Statistically, there was no significant difference among all metal tested in onions samples from suppliers 1, 2, 3, 4 and 5. The metal concentration in onions were in an ascending order of Sr > Mn > V > As > Cr > Cu > Ba > Rb > Ni > Co as shown in Table 2.

Estimated daily intake of metal (DIM)

The estimated DIM of As and Cr concentrations in

spinach were higher than those recommended by FAO/WHO obtained from all suppliers, while, Ba, Cu, Co, Mn, Sr and V were below the recommendation for FAO/WHO (Table 3).

The estimated DIM for As concentration in onion were higher than those recommended by FAO/WHO from all suppliers. The estimated DIM for Ba, Cr, Cu, Mn, Ni, Sr and V concentrations in onions were below the recommendation of FAO/WHO. Ba and Sr are unessential elements therefore not recommended as given by FAO/WHO (Table 3).

As and Cr are naturally occurring in soil as earth elements. Arsenic can also results from pesticide, herbicide and fungicide (Gilbert, 2004), used to protect the vegetables from pest, herbs and fungi during the production stage. Exposure to inorganic arsenic is primarily of concern because of its cancer-causing properties. Arsenic has been classified by the International Agency for Research into Cancer (IARC) as a human carcinogen on the basis of increased incidence of cancers at several sites in people exposed to arsenic at work, in the environment or through their diet (ATSDR, 2005). However, arsenic is also more acutely toxic than other metallic compounds and was used in earlier times as a rodenticide, while continual low level exposure to arsenic is associated with skin, vascular and nervous system disorders (FSAI, 2009).

Target hazard quotient (THQ)

The THQ for As, Ba, Cu, Co, Mn, Ni, Ti, Sr and V for spinach obtained from suppliers 1, 2, 3, 4 and 5 were all below WHO benchmark 1 as shown in Table 4. All THQ of less than 1 indicates that the chemical exposure is unlikely to results in adverse effect (WHO, 2010). However, the THQ for Cr was found to be 2.19 in spinach obtained from supplier 5. This indicates that the Cr exposure is likely to result in adverse effect (WHO, 2010). THQ value for Cr in spinach obtained



Figure 1. Southern Africa map showing Mafikeng* (Encyclopaedia Britannica, 2009).

Table 2. Metal concentration in spinach and onion from suppliers 1, 2, 3, 4 and 5 (mean \pm SDTV).

Metal	Spinach (mg/g)					Onions (mg/g)				
	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5
As	0.017 \pm 0.004	0.020 \pm 0.004	0.021 \pm 0.005	0.028 \pm 0.006	0.017 \pm 0.005	0.025 \pm 0.005	0.020 \pm 0.004	0.028 \pm 0.006	0.047 \pm 0.007	0.020 \pm 0.004
Ba	0.009 \pm 0.001	0.007 \pm 0.001	0.004 \pm 0.000	0.005 \pm 0.001	0.006 \pm 0.001	0.001 \pm 0.000	0.004 \pm 0.001	0.001 \pm 0.001	0.004 \pm 0.001	0.005 \pm 0.001
Cr	0.016 \pm 0.002	0.009 \pm 0.001	0.020 \pm 0.002	0.005 \pm 0.001	1.150 \pm 0.088	0.004 \pm 0.000	0.003 \pm 0.001	0.021 \pm 0.002	0.005 \pm 0.001	0.003 \pm 0.001
Cu	0.006 \pm 0.001	0.009 \pm 0.001	0.003 \pm 0.000	0.005 \pm 0.001	0.003 \pm 0.001	0.004 \pm 0.001	0.006 \pm 0.001	0.002 \pm 0.001	0.010 \pm 0.001	0.005 \pm 0.001
Co	0.001 \pm 0.000	0.001 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.001	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000
Mn	5.884 \pm 0.543	2.636 \pm 0.178	0.746 \pm 0.070	1.740 \pm 0.150	0.644 \pm 0.051	0.017 \pm 0.002	0.460 \pm 0.032	0.030 \pm 0.003	0.027 \pm 0.001	0.019 \pm 0.002
Ni	0.001 \pm 0.000	0.000 \pm 0.000	0.004 \pm 0.001	0.000 \pm 0.000	0.002 \pm 0.001	0.002 \pm 0.000	0.005 \pm 0.001	0.006 \pm 0.001	0.001 \pm 0.000	0.001 \pm 0.000
Sr	0.515 \pm 0.060	0.009 \pm 0.001	0.015 \pm 0.001	0.014 \pm 0.002	0.020 \pm 0.002	0.011 \pm 0.001	0.013 \pm 0.001	0.462 \pm 0.056	0.034 \pm 0.001	0.676 \pm 0.079
V	0.036 \pm 0.004	0.043 \pm 0.003	0.046 \pm 0.004	0.059 \pm 0.006	0.039 \pm 0.006	0.061 \pm 0.006	0.047 \pm 0.004	0.066 \pm 0.007	0.085 \pm 0.010	0.044 \pm 0.004

Table 3. Estimated DIM in spinach and onions.

Metal	Spinach					Onions					FAO/WHO mg/kg body weight per day
	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5	
As	0.099	0.116	0.122	0.160	0.099	0.141	0.116	0.162	0.269	0.112	0.020
Ba	0.053	0.042	0.023	0.030	0.036	0.006	0.025	0.008	0.025	0.027	-
Cr	0.090	0.053	0.112	0.030	6.571	0.023	0.019	0.122	0.030	0.019	0.033
Cu	0.032	0.051	0.017	0.030	0.019	0.025	0.036	0.013	0.055	0.030	2-3
Co	0.006	0.006	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	-
Mn	33.621	15.065	4.261	9.941	3.680	0.099	2.630	0.171	0.156	0.109	2-5
Ni	0.006	0.000	0.021	0.000	0.013	0.011	0.030	0.036	0.006	0.006	0.100
Sr	2.945	0.051	0.084	0.082	0.116	0.063	0.076	2.638	0.194	3.865	-
V	0.208	0.246	0.261	0.339	0.221	0.349	0.270	0.379	0.484	0.253	10

FAO/WHO- Food and Agriculture Organization/World Health Organization, 1996, bw- body weight.

Table 4. Target hazard quotient (THQ) of metals in spinach and onions.

Metal	Spinach					Onions				
	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5	Supplier 1	Supplier 2	Supplier 3	Supplier 4	Supplier 5
As	3.30×10^{-01}	3.87×10^{-01}	4.06×10^{-01}	5.33×10^{-01}	3.30×10^{-01}	4.70×10^{-01}	3.87×10^{-01}	5.40×10^{-01}	8.95×10^{-01}	3.75×10^{-01}
Ba	2.67×10^{-04}	2.09×10^{-04}	1.14×10^{-04}	1.52×10^{-04}	1.81×10^{-04}	2.86×10^{-05}	1.24×10^{-04}	3.81×10^{-05}	1.24×10^{-04}	1.33×10^{-04}
Cr	2.98×10^{-02}	1.78×10^{-02}	3.75×10^{-02}	1.02×10^{-02}	2.19	7.62×10^{-03}	6.35×10^{-03}	4.06×10^{-02}	1.02×10^{-02}	6.35×10^{-03}
Cu	8.10×10^{-04}	1.29×10^{-03}	4.29×10^{-04}	7.61×10^{-04}	4.76×10^{-04}	6.19×10^{-04}	9.05×10^{-04}	3.33×10^{-04}	1.38×10^{-03}	7.62×10^{-04}
Co	1.90×10^{-02}	1.90×10^{-02}	0.00	0.00	6.29×10^{-03}	0.00	0.00	0.00	0.00	0.00
Mn	2.40×10^{-01}	1.08×10^{-01}	3.04×10^{-02}	7.10×10^{-02}	2.63×10^{-02}	7.07×10^{-04}	1.88×10^{-02}	1.22×10^{-03}	1.12×10^{-03}	7.76×10^{-04}
Ni	2.86×10^{-04}	0.00	1.05×10^{-03}	0.00	6.66×10^{-04}	5.71×10^{-04}	1.52×10^{-03}	1.81×10^{-03}	2.86×10^{-04}	2.86×10^{-04}
Sr	4.91×10^{-03}	8.57×10^{-05}	1.40×10^{-04}	1.36×10^{-04}	1.94×10^{-04}	1.05×10^{-04}	1.27×10^{-04}	4.40×10^{-03}	3.24×10^{-04}	6.44×10^{-03}
V	4.15×10^{-02}	4.91×10^{-02}	5.22×10^{-02}	6.78×10^{-02}	4.42×10^{-02}	6.97×10^{-02}	5.41×10^{-02}	7.58×10^{-02}	9.68×10^{-02}	5.07×10^{-02}

from suppliers 1, 2, 3 and 4 were less than 1 which is unlikely to result in adverse effect. The THQ for As, Ba, Cr, Cu, Mn, Ni, Ti, Sr and V for onions obtained from suppliers 1, 2, 3, 4 and 5 were all below WHO benchmark of 1 as shown in Table 4 (WHO, 2010).

Thus, this study only measure total Cr, it should be noted that Cr (III) toxicity occurs in higher concentrations, and this form is actually an essential nutrient to human and other animals. Cr (VI), on the other hand, is toxic in much lower concentrations and also tends to be more mobile and bioavailable than Cr (III) in surface and subsurface environments (Adriano, 2001).

When compared with other pathways such as inhalation and dermal contact, dietary intake is the main route of exposure to heavy metals for most people (Tripathi et al., 1997; Qian et al., 2010; Yeganeh et al., 2012). Vegetables take up heavy metals such as Cr and accumulate them in their edible and non-edible parts at quantities high enough to cause clinical problems to both animals and human beings. As an example, the consumption of contaminated food can seriously deplete some essential nutrients in the body causing a decrease of immunological defenses, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Oliver, 1997).

Conclusion

The results of the study show successful detection of As, Ba, Cu, Cr, Co, Mn, Ni, Pb, Sr, and V concentrations in spinach and onions. Metals can naturally occur in soil and also as a result of human activities and end up accumulating in vegetables as mineral. Depending on metal concentration, the mineral can be regarded as contaminants with potential human health effect.

The THQ value for As in both spinach and onions were below 1, while the THQ for Cr was greater than 1 which signified that a daily exposure of Cr at this level is likely to cause adverse effects during a person lifetime, Cr was proven to be carcinogenic to humans in the study that was listed in the First Annual Report on Carcinogens in 1980 by US National Toxicology Program.

The study also found that As and Cr daily intake in spinach and onions were higher than that recommended by FAO/WHO from all suppliers. The community should not ignore the estimated DIM for As that was detected in spinach and onions.

Due to metal concentration, the vegetables are not suitable for human consumption and should be consumed with caution because they might cause health effect if the metal concentration remains at the same level or higher. Further study should be conducted to ascertain the sources of As and Cr detected in the spinach and onion.

CONFLICT OF INTERESTS

The authors did not declare any conflict of interest.

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REFERENCES

- Adriano DC (2001). Trace Elements in Terrestrial Environments. 2nd Edition. Springer-Verlag, New York, NY, USA.
- Agency for Toxic Substances and Disease Registry (ATSDR) (2005). Toxicological profile for tin and tin compounds. US Department of health and human services. Public Health Service.
- Encyclopaedia Britannica (2009). North West. North West province, S.Af. Encyclopaedia Britannica, Available at: <https://www.britannica.com/place/North-West-province-South-Africa>
- Environmental Affair, Republic of South Africa (EA-RSA) (2010). Framework for Environmental management of Contaminated Land. P 23.
- Food Safety Authority Ireland (FSAI) (2009). Mercury, Lead, Cadmium, Tin and Arsenic in Food. Toxicology factsheet series. P 1.
- Ghosh AK, Bhatt MA, Agrawa HP (2012). Effect of longterm application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. Environ. Monit. Asses. 184:1025-1036.
- Gilbert SG (2004). A Small Dose of Toxicology: The Health Effects of Common Chemicals, 1st Edition, CRC Press LLC. ISBN:978-0-9833378-3-6.
- McGrath MT (2004). What are Fungicides. The Plant Health Instructor. Available at: <http://www.apsnet.org/edcenter/intropp/topics/Pages/Fungicides.aspx>
- Nordberg GF, Fowler BA, Nordberg M (eds) (2007). Handbook on the Toxicology of Metals. 3rd edition. Elsevier. ISBN.978-0-12-369413-3.
- Nordberg GF, Fowler Monica BA, Nordberg M (eds) (2015). Handbook on the Toxicology of Metals. 4th Edition, In: Alison Elder, Gunnar T Nord Berg and Michael Kleinman. Routes of Exposure, Dose, and Toxicokinetics of Metal. pp. 1-48.
- Oliver MA (1997). Soil and human health: a review. Eur. J. Soil Sci. 48:573-592.
- Orisakwe OE, Nduka JK, Amadi CN, Dike DO, Bede O (2012). Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. Chem. Cent. J. 6(1):77.
- Qian YZ, Chen C, Zhang Q, Li Y, Chen ZJ, Li M (2010). Concentrations of cadmium, lead, mercury and arsenic in Chinese market milled rice and associated population health risk. Food Control 21(12):1757-1763.
- Tripathi RM, Raghunath R, Krishnamoorthy TM (1997). Dietary intake of heavy metals in Bombay city, India. Sci. Total Environ. 208:149-159.
- United State Environmental Protection Agency (USEPA) (2015). Human Health Risk Assessment. Available at: <https://www.epa.gov/risk/human-health-risk-assessment>.
- United State Environmental Protection Agency (USEPA) (2016). USEPA Regional Screening Level (RSL) Summary Table. Available at: <https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables-may>.
- United States Environmental Protection Agency (USEPA) (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis 3rd Edition, Office

- of Science and Technology of Water, Washington, DC EPA, 823-B-00-007:1-200.
- Wang X, Sato T, Baoshan X (2005). Health risk of heavy metals to the general public of Tianjin, China via consumption of vegetables and fish. *Sci. Total Environ.* 350:28-37.
- World Health Organization (WHO) (1996). Trace elements in human nutrition and health.
- World Health Organization (WHO) (2010). Geneva World Health Organization WHO Human health risk assessment toolkit: Chemical hazards. P 8.
- Yeganeh M, Afyuni M, Khoshgoftarmanesh AH, Soffianian AR, Schulin R (2012). Health risks of metals in soil, water, and major food crops in Hamedan Province, Iran. *Human and Ecological Risk Assess.* Int. J. 18(3):547-568.
- Zukowska J, Biziuk M (2008). Methodological evaluation of method for dietary heavy metal intake. *J. Food Sci.* 73(2):R21-R29.