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Preliminary investigation of transfer of metals from soil to vegetables: Case study of *Spinacia oleracea* L.

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The objective of the study was to measure concentrations of Cu, Ni and Zn in *Spinacia oleracea* cultivated at a site near the copper and nickel mine in Selebi Phikwe. The mean concentrations (in dry matter-basis) of Cu, Zn and Ni in the whole plant system were 7.30 ± 2.51, 6.02 ± 2.16 and 0.03 ± 0.02, mg/kg, respectively. Enrichment factors (EF) of Cu, Ni and Zn were far below the EF value of 1.5 suggesting that the soils at the study site were either good in retention of metals and/or there was minimal translocation of metals in the plants. The authors recommend a multiple exposure effect of heavy metal monitoring to be conducted regularly at the study site.

Key words: Dietary toxicity, estimated dietary intake, *Spinacia oleracea*, target hazard quotient.

INTRODUCTION

Deteriorating environmental conditions resulting from urban industrial activities such as mining and agricultural practices such as application of phosphate fertilisers and sewage sludge are the main man made sources of metals in agricultural soils (Nriagu, 1990; Alloway and Jackson, 1991). Soil-to-plant transfer of heavy metals is the major pathway of human exposure to soil contamination (Cui et al., 2004). For example, studies in Japan (Ryan et al., 1982) and China (Cai et al., 1990; Jin et al., 2002) have reported that lifetime exposure to low level soil contamination with cadmium (Cd), caused renal dysfunction in residents living near the contaminated sites of the respective study areas.

Vegetables are rich sources of vitamins, minerals, fibres and also have antioxidative effects (Jena et al., 2012). Thus, heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of the human diet. Heavy metal concentrations in edible parts of plants is directly associated with their concentrations in soils, but their levels differ significantly with plant species, and sometimes with the genotypes within the same plant species (Kabata-Pendias and Pendias, 1984). Furthermore, plants display a wide range of adaptations to soils with contrasting metal contents. Heavy metal uptake and translocation are key aspects of plants' ability to accumulate and cope with high concentrations of heavy metals.

Studies by Akan et al. (2013) from four agricultural soils in Nigeria showed higher concentrations of lead (Pb), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), nickel (Ni),

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manganese (Mn) and chromium (Cr) in the leaves of spinach, lettuce, cabbage and onions than their roots and stems. Ingestion of vegetables containing heavy metals is one of the main ways in which these elements enter the human body, besides water, dust and several other pathways. Once entered, the metals are deposited in the bone and fat tissues and can cause a range of diseases.

On sensory evaluation basis, dark, green, big leafy vegetables appear safe and of good quality to most consumers, but this alone cannot guarantee that the vegetables are safe for consumption. The importance of vegetables health-wise has led to an increasing demand for vegetables. As such, many people in semi-urban areas of Botswana including the study area in Selebi Phikwe are involved in urban agriculture. Selebi-Phikwe is a copper and nickel mining town of Botswana. Mining and smelting activities have been practiced for more than three decades and are at present associated with environmental problems that include soil, water and air pollution (Schwartz and Kgomanyane, 2008; Ekosse, 2005, 2011; Likuku et al., 2013).

In Botswana, literature is scares regarding transfer of metals and their potential effects to the consumers. Because peri-urban agriculture is practiced in Selebi Phikwe to meet the vegetable demands of the local population, environmental surveys including possible heavy metal contamination is of increasing concern about the safety of crops grown around that area since soil to plant transfer of heavy metals is one of the key components of human exposure to metals through the food chain (Li et al., 2006). The present study was conducted with the aim to (1) quantify the content of Cr, Cd, Cu, Ni and Zn in *Spinacia oleracea* L. (spinach) grown around the copper nickel mining and smelter sites of Selebi Phikwe in Botswana, (2) to evaluate the biological concentration factors, translocation factors and biological accumulation coefficients so as to investigate the degree of pollution and daily intake amount of metals through consumption of the vegetable and finally (3) assess potential health risk to local population via oral exposure routes.

MATERIALS AND METHODS

Sample collection and preparation

The study was conducted at horticultural farms near the Selebi Phikwe copper and nickel mine and smelter area (21.98°S; 27.84°N). The farms were approximately 15 km from the mines. At these farms, *S. oleracea* L. and soils were collected from different sites of the farms. As a reference uncontaminated area, the same sampling procedures were conducted in a farm approximately 60 km away from the mines in the same district where soils are similar to those of the contaminated areas, but without a comparable source of metal contamination. Both farms were drip irrigated. A map showing the position of the experimental area is presented in Figure 1.

Plant and soil sampling

Three planted plots of *S. oleracea* L. were systematically selected to cover the whole farm at the Selebi Phikwe copper and nickel mine and smelter experimental site. From each plot, three recently matured edible parts of spinach were uprooted. Soils from the sampled plots were also sampled at root level, approximately 0–30 cm, using stainless steel hand corers.

For the purpose of experimental control, the same vegetable type was also collected in triplicates from a farm approximately 60 km away to mimic pristine sites. Soil samples were also collected as before. Thus, a total of 18 vegetable and 18 soil samples were collected from both farms. All the samples were brought in polythene bags to the Botswana College of Agriculture laboratories for analysis.

Sample preparation

In the laboratory, plant samples were double rinsed with deionised water to remove adhered soil and dust particles before being sliced into small pieces. The spinach was sectioned into three different compartments: the edible part (the leaves), the stem and the roots. Both plant and soil samples were then oven dried at 50°C until weight constancy, homogenised using a pestle and mortar and then passed through a 2 mm stainless sieve and stored at room temperature for further analysis.

For heavy metal extraction, approximately 0.5 g of dried ground samples were digested with 6 ml of 65% nitric acid (HNO_3) and 2 ml of 30% hydrogen peroxide (H_2O_2) (with 3 ml of 65% HNO_3 and 9 ml of 37% H_2O_2 for soils) in Ethos EZ Microwave Digestion System from Milestone following the condition described: Maximum Power–15000 W; Ramp time– 110 min; hold time– 110 min; temperature–200°C.

After hold time, the vessels were allowed to cool for about 30 min. The suspension was filtered (Whatman filter Merck, 0.45 µm) and the filtrate was diluted by deionized water to a final volume of to 100 ml with deionised water and stored in a refrigerator (<4°C) until analysis (Allen et al., 1986).

Metal analysis

Levels of Cu, Ni and Zn in the solutions were measured using Perkin-Elmer Spectrophotometer Model 460 flame atomic absorption spectroscopy (FAAS) at the Department of Waste Management and Pollution Control, in Gaborone, Botswana. The AAS was optimised and a calibration curve was produced using standards prepared from pipettes of 5 ml each of the 1000 mg/L stock solutions of Cu, Mn, Cd, Co, Al, Pb, Zn, Ni, Mg; 10 ml Fe stock solutions and 20 ml Cr stock, five micrograms per millilitre (5 µg/mL) for Cu, Mn, Cd, Co, Al, Pb, Zn, Ni, Mg; 10 µg/mL for Fe; and 20 µg/ml for Cr. Calibration curves were then prepared using the stock solutions. The instrumental parameters were set depending on the type of analysis done. Samples were aspirated in triplicates.

Data analysis

Biological concentration factor (BCF) was calculated as metal concentration ratio of plant roots to soil given in Equation 1 (Yoon et al., 2006). Translocation factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root given in Equation 2 (Cui et al., 2007; Li et al., 2007). Biological accumulation coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil given in Equation 3 (Li et al., 2007; Cui et al., 2007):

Figure 1. Map of Botswana showing Selebi Phikwe (the star with coordinates indicated). The maps are not drawn to scale.

$$BCF = \left(\frac{[Metals]_{root}}{[Metals]_{soil}} \right)_{Dry\ Weight}$$
………………………………………… (1)

$$TF = \left(\frac{[Metals]_{shoot}}{[Metals]_{root}} \right)_{Dry\ Weight}$$
………………………………………… (2)

$$BAC = \left(\frac{[Metals]_{shoot}}{[Metals]_{soil}} \right)_{Dry\ Weight}$$
………………………………………… (3)

Statistical analysis

Basic statistical analysis of maximum, minimum mean and standard deviation values were performed to compare levels of heavy metals in different components of *S. oleracea* at experimental (E) sites as well as at the control (*C*) site.

Normalisation procedure

To analyze anthropogenic enrichment, enrichment factor, *EF* was used to geochemically normalise the dataset and ascertain experimental control or background relationships between pristine site and the site of concern. The enrichment factor was calculated using the formula originally introduced by Buat-Menard and Chesselet (1979) as shown in Equation 4:

$$EF = \left(\frac{\left(C_{plant}/C_{soil}\right)_{Experimental\ site}}{\left(C_{plant}/C_{soil}\right)_{Control\ site}} \right)_{Dry\ Weight}$$
………………………………………… (4)

Where C_{plant} is the edible plant material content and C_{soil} is the total material content in soil where the plant was grown at both the wastewater and groundwater irrigated sites, all expressed in dry weight. It is worth noting that the ratios $C_{\text{plant}}/C_{\text{soil}}$ symbolise translocation of metals in plants.

Enrichment factor categories proposed by Sutherland (2000) was then used as follows: *EF* < 2 = deficiently to minimal enrichment, 2 ≤ *EF* < 5 = moderate enrichment, 5 ≤ *EF* < 20 = significant enrichment, 20 ≤ *EF* < 40 = very high enrichment and EF ≥ 40 = extremely high enrichment. For this work, metal enrichment will be considered when *EF* ≥ 1.5, symbolising minimal enrichment and above.

Statistical analysis

The risk to human beings resulting from consumption of spinach grown from the area was calculated by employing the estimated dietary intake (*EDI*, in mg/kg-per person per day) and target hazard quotient (*THQ*) described by Zheng et al. (2007) and USEPA (1989) as shown in Equation 5:

$$EDI = \frac{C_{\text{plant}}(\text{mg/kg}) \times \text{Intake}\,(\text{kg/person/day}) \times EFr \times ED}{BM\,(\text{kg}) \times AT} \quad \ldots\ldots (5)$$

In Equation 5, C_{plant} is the median concentration of heavy metal in spinach, intake is the ingestion rate, *EFr* is the exposure frequency given as 350 days/year (Wang et al., 2012), *ED* is the exposure duration. In this work, it was proposed that *ED* will be 30 years for adults (Grzetic and Ghariani, 2008), and *AT* is the average time for non-carcinogens of 365 days/year (USEPA, 2005). The average adult daily vegetable intake rate of 0.345 kg/person/day and body mass of 55.9 kg was used as reported in the literature (Ge, 1992; Wang et al., 2005). The conversion factor 0.085 was used to convert fresh green vegetable weight to dry weight, as described by Rattan et al. (2005). This gives the total dose entering the human body through oral ingestion of contaminated vegetables.

Target hazard quotient, *THQ* was determined from the ratio of the *EDI* to oral reference dose RfD_o values obtained from Integrated Risk Information Systems (IRIS, 2003) and the Department of Environment, Food and Rural Affairs (DEFRA, 1999) given in Equation 6:

$$THQ = \frac{EDI}{RfD_o\ (\text{mg/kg per person per day})} \quad \ldots\ldots (6)$$

In order to assess the overall potential for non-carcinogenic effects, the total chronic hazard index, *THI* which is the summation of all the individual target hazard quotients is represented as in Equation 7:

$$THI = \sum_{i=1}^{n} THQ \quad \ldots\ldots (7)$$

If either value of *THQ* and *THI* are above 1, a high risk of non-carcinogenic effects is implied, since the accepted standard is 1.0 at which there will be no significant health hazard (Grzetic and Ghariani, 2008; Lai et al., 2010). The probability of experiencing long-term health hazard effects increases with the increasing *THI* value (Wang et al., 2012) and according to Lemly (1996); *THI* = 1.1–10 refers to moderate hazard while *THI* >10 refers to high hazard.

RESULTS AND DSCUSSION

Concentration of metals

Mean concentrations of Cu, Zn and Ni in whole plan system (leaf + stem + root) at the experimental site were 7.30 ± 2.51, 6.02 ± 2.16 and 0.03 ± 0.02 mg/kg, respectively. Control site mean concentrations of Cu and Zn were 4.01 ± 3.26 and 4.11 ± 1.94 mg/kg, respectively, whereas Ni concentrations at the control site were below detection. Experimental site mean concentrations of Cu and Zn in soils were significantly high; $p = 0.014$ and $p = 0.033$, respectively, as compared to those measured at the control site. Similarly, the mean concentrations of Cu (24.37 ± 0.04 mg/kg) and Zn (12.97 ± 0.21 mg/kg) at the experimental site were significantly higher than those at the control sites: Cu (3.23 ± 0.27 mg/kg) and Zn (7.08 ± 1.17 mg/kg) at $p = 0.0005$. On the leaf-stem-root compartmental basis, the mean concentrations of Cu, Zn, Ni, Cd, Cr and Pb in *S. oleracea* and in soils from experimental and control sites are given in Table 1.

The maximum permissible concentration of Cu in plants recommended by the World Health Organisation (WHO) is 73 mg/kg (Chiroma et al., 2012). From the results obtained in this study, Cu was found to be lower in the edible part of spinach (leaf) than the permissible limit by approximately 6%. The mean copper content in the cultivated soils from this experiment was found to be 23.90 ± 3.31 mg/kg. This value is within the normal Cu content of 5–50 mg/kg (Mico, 2006) and far below the maximum permissible values of 100 mg/kg for Cu in horticultural soils (Kabata-Pendias, 2001). Copper is an essential micronutrient involved in a number of biological processes needed to sustain life. However, it can be toxic when present in excess (de Romañ et al., 2011).

In the case of Zn, the amount measured in the edible part of spinach was found to be 6.02 ± 2.16 and 12.97 ± 0.21 mg/kg in the respective cultivated soil. These values are below the WHO's recommended limit of 50 mg/kg for zinc in plants and the 300 mg/kg recommended limit values reported in Kabata-Pendias and Pendias (2001). Zinc is one of the most important trace elements that play a vital role in the physiological and metabolic processes of many organisms. Traces of Ni at << 0.1 mg/kg were recorded in the plant system, but not in soils. The rest of the measured elements: Pb, Cr and Cd were below the instrument detection limits of 0.0045, 0.0054 and 0.014 mg/kg, respectively.

Transfer of metal from soil to plant is the major pathway of exposure to humans through the food chain. This was measured though enrichment from soils to the plant system using Equation 4, based on values obtained from Equations 1 to 3. As shown in Tables 2 and 3, it is evident from the results obtained and presented in Table 1 that enrichment of metals was below unity. As stated earlier, metal enrichment will be considered only when

Table 1. Statistics of metal concentrations (in mg/kg-dry matter) showing the minimum (Min), maximum (Max), mean values and their corresponding standard deviations (SDEV).

	Cu		Zn		Ni		Cd		Cr		Pb	
	E	***C***	***E***	***C***	***E***	***C***	***E***	***C***	***E***	***C***	***E***	***C***
(N = 9)	**Leaf component at experimental (*E*) control (*C*) sites**											
Min.	1.87	1.82	4.52	4.37	0.00	ND	ND	ND	ND	ND	ND	ND
Max.	6.53	8.10	7.22	6.96	0.02	ND	ND	ND	ND	ND	ND	ND
Mean	**4.73**	**4.72**	**5.59**	**6.02**	**0.01**	–	–	–	–	–	–	–
SDEV	2.05	2.59	1.17	1.17	0.01	–	–	–	–	–	–	–
(N = 9)	**Stem component at experimental (*E*) control (*C*) site**											
Min.	7.11	0.58	4.28	1.29	0.01	ND	ND	ND	ND	ND	ND	ND
Max.	9.67	10.40	5.90	3.96	0.02	ND	ND	ND	ND	ND	ND	ND
Mean	**8.20**	**4.85**	**5.21**	**2.49**	**0.02**	–	–	–	–	–	–	–
SDEV	1.08	4.11	0.68	1.11	0.00	–	–	–	–	–	–	–
(N = 9)	**Root component at experimental (*E*) control (*C*) site**											
Min.	7.66	1.17	4.82	2.33	0.04	ND	ND	ND	ND	ND	ND	ND
Max.	10.40	3.74	11.30	4.73	0.04	ND	ND	ND	ND	ND	ND	ND
Mean	**8.98**	**2.45**	**7.27**	**3.81**	**0.04**	–	–	–	–	–	–	–
SDEV	1.12	1.05	2.87	1.06	0.00	–	–	–	–	–	–	–
(N = 9)	**Soil component at experimental (*E*) control (*C*) site**											
Min.	23.90	2.93	12.20	6.08	0.05	ND	ND	ND	ND	ND	ND	ND
Max.	24.60	3.46	13.20	8.40	0.06	ND	ND	ND	ND	ND	ND	ND
Mean	**24.37**	**3.23**	**12.97**	**7.08**	**0.05**	–	–	–	–	–	–	–
SDEV	0.33	0.22	0.17	0.97	0.00	–	–	–	–	–	–	–

ND: Not detected.

Table 2. Biological concentration factors, transfer factors and bioaccumulation factors of metal concentrations between soils and the vegetable plant at the experimental site.

	Cu	Zn	Ni
BCF	0.37	0.53	0.75
TF	0.56	0.77	0.25
BAC	0.75	0.43	0.19

BCF = Biological concentration factor; TF = translocation factor; BAC = biological accumulation coefficient.

Table 3. Normalised enrichment factors, *EF* of Cu and Zn in the leaf, stem and roots of *Spinacia oleracea.*

	Leaf	Stem	Root
Cu	0.133	0.224	0.486
Zn	0.510	1.141	1.041

EF values are greater than 1.5. Lower values of heavy metal enrichment factors obtained in this study suggest good retention of metals in soils and/or less translocation in plants.

Potential health risk

Health risks to residents in the study area through consumption of *S. oleracea* were assessed by target hazard quotient *THQ* obtained by estimating the dietary intake (*EDI*) expressed using Equation 5. Table 4 summarises the exposure assessment for adults in the study area.

The applied non-carcinogenic oral reference dose RfD_o values obtained from Integrated Risk Information Systems (IRIS, 2003) for Cu and Zn were respectively 0.04 and 0.3 mg/kg/d. The *EDI* values for Cu and Zn were 0.10 and 0.08 mg/kg BM/d, respectively. The *EDI* values for Cu in *S. oleracea* is approximately 2.5 times higher than the RfD_o value suggesting that people consuming the spinach cultivated from the study site may

Table 4. Estimated daily intake, *EDI* in mg/kg BM/d, and target hazard quotient, *THQ* for adults through consumption of *Spinacia oleracea* from the experimental site.

	EDI	THQ	
Cu	0.10	2.44	ΣTHQ = THI
Zn	0.08	0.27	
Total	0.18	2.71	2.89

experience adverse effects due to the presence of Cu in the vegetable, whereas Zn was about 0.22 mg/kg BM/d below its RfD_o value.

The total *THQ* (the health risk index, *THI*) for both Cu and Zn was 2.89. According to Lemly (1996), *THI* = 1.1–10 refers to moderate hazard. It was difficult to reconcile these values with others studies from Botswana since literature on heavy metal exposure and assessment on their effects to human health was unavailable. However, as compared to other countries, the value obtained in this study was relatively high; for example, multiple-exposure of *THQ* values for Cu and Zn from independent studies at a mining area in China were found to be 2.1 (Zhuang et al., 2014).

There is increasing evidence worldwide that pollution due to heavy metal contaminants from mining area cause death, for example, in Zambia alone, tens of thousands of the residents of Kwabe suffered from lead poising by the lead-zinc mining and smelting (Branan, 2008). In japan, release of the metal into the river water that was used for irrigation at the Kamioka mine was responsible for the itai-itai disease that was caused by Cd poising to the local people (Abrahams, 2002). Thus, monitoring of heavy metal concentration and exposure to humans and understanding the human exposure pathways would be of help in the possible need for intervention, and possible medical surveillance. It is recommended that monitoring of these metals must be done regularly at these sites, not only for spinach but all the commonly edible plants vegetables and other foodstuff including ingestion through drinking water, so as to provide a better picture of the levels of the *THI* for multiple exposure pathways.

Conclusions

Concentrations of Cd, Cr, Cu, Ni, Pb and Zn were measured in soils and in *S. oleracea* cultivated at a site near the copper and nickel mine in Selebi Phikwe. The mean concentrations of Cu, Zn and Ni in whole plan system (leaf + stem + root) at the experimental site were 7.30 ± 2.51, 6.02 ± 2.16 and 0.03 ± 0.02 mg/kg, respectively. The mean concentrations of Cu in spinach and in the cultivated soils were below the WHO's permissible limits. Similarly, the mean concentrations of Zn were also found to be below the recommended limits for both plants and horticultural soils. Enrichment factors of Cu, Ni and Zn were far below the *EF* value of 1.5, suggesting that the soils at the study site are either good in retention of metals and/or there was minimal translocation of metals in the plants.

Conflict of interest

The authors have not declared any conflict of interest.

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