Health Risk Assessment of Heavy Metals via Dietary Intake of Vegetables Irrigated With Treated Wastewater around Gaborone, Botswana

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***Abstract*—**Heavy metal concentrations of Cd, Cr, Cu, Ni, Pb and Zn in green peppers (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) irrigated with treated wastewater from the Glen Valley farms were determined. Concentrations of Cr, Pb and Zn were significantly high when compared with their respective recommended safe limits prescribed by the Joint WHO/FAO Food Standards Program Code Alimentarius Commission. The daily intake of metals, *DIM* values for Cr and Pb were also found to be higher than their recommended safe limit values, whereas those for Cd, Cu and Ni were within the safe limit values while that for Zn was far below the recommended safe limit value. In this study, we expect that consumption of Cr and Pb through vegetables poses substantial health risk to consumers and for this reason, these products are probably not recommended consumption. Therefore, this emphasizes the need for proper method to manage using wastewater to reduce the health risk and the extent of heavy metals contamination.

***Keywords*—**Cluster Analysis, Daily Intake of Metals, Health Risk Index, Normalised Enrichment Factors

I. Introduction

VEGETABLES constitute an important part of human diet since they contain carbohydrates, proteins, vitamins, minerals as well as trace elements. They also act as neutralizing agents for acidic substances formed during digestion [1]. Perceptions of what is regarded as 'better quality vegetables' are however subjective. Some consumers consider undamaged, dark green and big leaves as characteristics of good quality leafy vegetables, however, the external morphology of vegetables cannot alone guarantee safety from contamination.

Population boom and urbanization have led to increasing demand for vegetables in cities and big settlement areas. This has resulted in urban agriculture, which plays an important role in providing vegetables and other agriculture produce for the dwellers, whereby it is often associated with wastewater usage because of fresh water scarcity. The use of treated wastewater is particularly common to farming communities in arid and semi-arid countries, examples of which include Botswana. This practice leads to accumulation of potentially toxic heavy metals in soils and ultimate uptake by crops and transfer up the food-chain or is leached to the ground water [2]. Generally, heavy metals can occur in soils in minute quantities but can accumulate in agricultural soils from various sources such as fertilizers, organic supplements, atmospheric deposition, wastewater irrigation and urban industrial activities [3]. A number of serious health problems can develop as a result of excessive dietary uptake of heavy metals through consumption of vegetables and other plants grown in areas of high anthropogenic pressure.

It should be pointed out though that under field conditions, it is very difficult to distinguish the source of one or more heavy metals that may be present in a particular vegetable crop since there are plenty of contaminating sources, and not all can be easily taken into account. Due to the dynamic nature of the environment, one cannot judge which chemical processes that may be taking place is solely responsible for uptake of heavy metals in crops. For example, (i) the presence of heavy metals in wastewater used for irrigation does not necessarily mean that they may accumulate in the soil in plant-available form; (ii) there will be significant differences from farm to farm depending on application of the wastewater from year to year; (iii) the presence of total quantities of heavy metals in the soil does not necessarily indicate uptake by the crops, as different metals are differently available [4] and there are differences in uptake and even tolerance between crop species [5]–[6]; (iv) there could be different background levels in soils due to different pedogenic activities and finally, (v) other sources of heavy metals could possibly be due to sources such as vehicular emissions.

The use of treated urban wastewater for irrigation is relatively recent in Botswana. Wastewater used for irrigation at the study area, the Glen Valley farms in Gaborone, is secondary treated. The wastewater is from the Gaborone sewage ponds, which is an effluent catchment from the city and its environs. The treatment process involves reducing of contaminants or growths that are left in the wastewater using biological treatment processes. Although this process effectively treats bacteria, or contaminant growth, it does not remove heavy metals from the wastewater. At the Glen Valley farms, the vegetables cultivated at include spinach (*Spinacia*

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oleracea L.), green peppers (*Capsicum annuum*) and tomato (*Solanum lycopersicum*). These crops are, after harvesting, sold to the local supermarkets, street hawkers and individuals for consumption. Although previous studies that have been carried out on use of secondary treated wastewater at the Glen Valley farms have reported negative results on possible adverse physical, chemical or biological effects on fruits and vegetables [7], there is still need for further investigation, particularly on the long term impact of the use of wastewater in the food chain..

The present study was undertaken to build up a comprehensive picture that sought to shed some light on the health and environmental risks of using urban wastewater in vegetable production, focusing particularly on accumulation of heavy metals in the food chain. The main objectives of this study were (1) to determine concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in soils and vegetables irrigated with treated wastewater at the Glen Valley farms; (2) assess and compare Translocation Factors (TF), leading to Enrichment Factors (EF) of heavy metals in green pepper (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) from treated wastewater with similar vegetables from groundwater irrigated farms in Ramotswa and (3) to highlight toxicology implications of heavy metals following risk assessment methods and suggest areas for further research.

II. Materials And Methods

A. Geographical Setting of the Vegetable Farms

The geographical location of the Glen Valley vegetable farms are (24.59–24.62)°S; (25.97–25.98)°E, situated on the eastern part of the city of Gaborone, along the A1-highway connecting the south and northern parts of Botswana (Fig. 1).

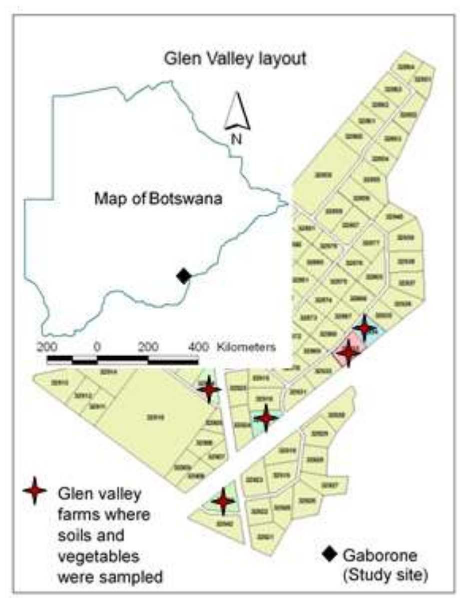

Fig. 1 Map of Botswana and the Glen Valley farm layout showing the five sites where soils and vegetables were sampled (the Botswana map and the Glen Valley layout are not shown to scale).

B. Plant and Soil Sampling

Five farms out the thirty two which are in use were systematically selected based on the availability of the vegetables at the Glen Valley farms. From each farm, recently matured edible parts of green peppers and tomatoes were sampled in sextuplicates. Soils were also sampled at root level (0–30) cm approximately, from the same farms using stainless steel hand corers. Three soil corers were collected from each vegetable plot, bulked according to vegetable type to represent one soil sample per plot (TABLE I).

TABLE I
Soil Sampling Strategies at Both the Glen Valley and the Ramotswa Farms

Farm/Sample	Glen Valley farms					Ramotswa	Totals
	A	B	C	D	E	Control	
Green-pepper	6	6	6	6	6	6	36
Tomatoes	6	6	6	6	6	6	36
Soils	3	3	3	3	3	3	18
Total	15	15	15	15	15	15	**90**

For the purpose of experimental control, the same vegetable types were also collected in sextuplicates from a groundwater irrigated farm in Ramotswa (site map not shown), to mimic pristine sites. Soil samples were also collected as before. Thus, a total of 72 vegetable and 18 soil samples (90 samples in total) were collected from both farms. All samples were brought in polythene bags to the Botswana College of Agriculture laboratories for analysis.

C. Sample Preparation

Plant samples were double rinsed with deionized water to remove adhered soil and dust particles before being sliced into small pieces. Both plants and soils samples were then oven dried at 70°C for three days, homogenized 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 0.3 g of dried ground samples were digested with 6 ml of 55% nitric acid (HNO_3) and 3 ml of hydrogen peroxide (H_2O_2) in ETHOS EZ microwave digester using the condition described below:

Maximum Power: 840 W
Ramp Time: 10 minutes
Hold Time: 30 minutes
Temperature: 160°C

After hold time, the vessels were allowed to cool for about 15 minutes. The digested solutions were then removed and made up to 50 ml with deionized water, kept in the refrigerator at 4°C in readiness for spectrometry analysis [8].

D. Heavy Metal Analysis

Levels of Cd, Cr, Cu, Ni, Pb and Zn in the solutions were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) at the Department of Agricultural Research laboratories, in Sebele. Standard reference materials were prepared using stock solution from SAARCHM and MERCH and used to have a check on the accuracy of the results.

Concentrations of these metals, Conc. (mg/kg), in samples were calculated following Uwah and Ogugbujaja [9] as shown in (1).

$$\text{Conc.}\left(\text{mg/kg}\right) = \frac{\text{Conc.}\left(\text{mg/l}\right) \times \text{Volume}, V}{\text{Sample mass}, M} \quad (1)$$

In (1), V is the final volume after digestion; M is the mass (in grams) of the sample to be tested and Concentration (mg/l) is the concentration of metal in digested solution.

E Statistical Analysis

Statistical tests of significance using *t*-tests and Analysis of Variance (ANOVA) to assess pairs of results in both soils and vegetables were performed.

To analyze anthropogenic enrichment, Enrichment Factor, *EF* was used to geochemically normalize 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 [10] defined in (2).

$$EF = \left(\frac{\left(C_{\text{plant}}/C_{\text{soil}}\right)_{\text{Glen Valley}}}{\left(C_{\text{plant}}/C_{\text{soil}}\right)_{\text{Control}}} \right)_{\text{Dry Weight}} \quad (2)$$

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 ground water irrigated sites, all expressed in dry weight. It is worth noting that the ratios $C_{\text{plant}}/C_{\text{soil}}$ symbolise translocation of bioaccumulation of metals in plants.

Enrichment factor categories proposed by Sutherland [11] were then used as follows: $EF < 2$ = deficiently to minimal enrichment, $2 \leq EF < 5$ = moderate enrichment, $5 \leq EF < 20$ = significant enrichment, $20 \leq \text{EF} < 40$ = very high enrichment and $EF \geq 40$ = extremely high enrichment. For this work, metal enrichment will be considered when $EF \geq 1.5$, symbolizing minimal enrichment and above.

Hierarchical cluster analysis using agglomerate schedule based on the nearest neighbor as an amalgamation rule and the Euclidean distance as a measure of the proximity between metal translocation in vegetable samples was performed. The metal translocation factors were determined from the ratios between the plants to soil heavy metal concentrations at the studied sites.

F Daily intake of metals (DIM)

The daily intake of metals (*DIM*) was assessed to estimate the average daily loading of metal into the body system of a specified body weight of a consumer. Although this does not take into account the possible metabolic ejection of the metals, it will however tell the possible ingestion rate of a metal in question. The daily intake of metal in this study was calculated based on the formula proposed by Sajjad et al. [12] shown in (3).

$$DIM = \left(\frac{C_{\text{plant}}(\text{mg/kg}) \times \text{Intake}\,(\text{kg/day})}{BM\,(\text{kg})} \right) \quad (3)$$

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 literature [13], [14].

G Health Risk Index (HRI)

The health risk index for Cd, Cr, Cu, Ni, Pb and Zn by consumption of contaminated vegetables were estimated from ratios of the *DIM* to oral reference dose *RfD* values shown in (4).

$$HRI = \frac{DIM}{RfD\,(\text{mg/kg/day})} \quad (4)$$

The oral reference dose values used in this study for Cd, Cr, Cu, Ni, Pb and Zn were 0.001, 0.003, 0.04, 0.02, 0.0035 and 0.3 (mg/kg body weight /day) respectively [15]. In this work, if the health risk index value was less than 1, then the exposed population was considered to be safe [16].

III. Results And Discussions

A. Mean Concentrations of the Studied Elements

Independent sample *t*–test data for Cd Cr, Cu, Ni, Pb, and Zn in green peppers and tomatoes from Glen Valley and the control sites are presented in TABLE II.

TABLE II
BASIC STATISTICAL DATA OF THE 6 ELEMENTS MEASURED IN THIS STUDY

Metal	Glen Valley Farms (mg/kg) (min–Max)	Glen Valley Farms (mg/kg) Mean (n = 60)	Control site (mg/kg) (min–Max)	Control site (mg/kg) Mean (n = 12)	*P*-value (2 tailed)
Metal concentrations in *Capsicum annuum* (green peppers)					
Cd	(0.01–1.36)	0.26 ± 0.22	(0.03–1.37)	0.72 ± 0.44	0.004
Cr	(0.37–55.63)	16.25 ± 12.24	(7.38–40.58)	21.88 ± 10.49	0.141
Cu	(0.74–477.46)	47.19 ± 96.58	(8.32–54.09)	31.42 ± 17.36	0.245
Ni	(1.71–15.01)	6.76 ± 3.28	(0.32–1.86)	1.00 ± 0.48	0.000
Pb	(7.22–23.77)	14.54 ± 3.99	(7.24–12.77)	10.83 ± 1.65	0.000
Zn	(37.32–196.94)	81.67 ± 32.35	(20.77–35.77)	28.51 ± 4.95	0.000
Metal concentrations in *Solanum lycopersicum* (tomatoes)					
Cd	(0.01–0.62)	0.19 ± 0.15	(0.18–3.12)	0.80 ± 0.80	0.019
Cr	(0.11–46.87)	15.34 ± 13.30	(3.22–41.66)	17.97 ± 12.55	0.531
Cu	(0.45–38.49)	7.88 ± 7.00	(0.58–3.23)	1.87 ± 0.99	0.000
Ni	(0.54–10.11)	2.67 ± 1.84	(0.08–1.62)	0.83 ± 0.46	0.000
Pb	(5.73–24.71)	13.68 ± 4.36	(7.36–15.34)	10.22 ± 2.54	0.010
Zn	(25.60–186.34)	78.75 ± 42.37	(21.30–75.22)	42.40 ± 16.72	0.000

An independent sample *t*–test was conducted to compare mean concentrations of the metals for the two vegetables irrigated with treated wastewater and those irrigated with ground water, as a control site. There were significantly higher the mean concentrations ($P < 0.05$) of Cd, Ni Pb and Zn in green peppers grown at the treated wastewater irrigated site (Glen valley) than the ground water irrigated (Ramotswa) control site. However, the mean concentrations of Cr (Mean = (16.25 ± 12.24) mg/kg) and (Mean = ((21.88 ± 10.49) mg/kg)); t (70) = –1.49, P = 0.14, and Cu (Mean = (47.19 ± 96.58) mg/kg) and (Mean = ((31.42 ± 17.36) mg/kg)); t (70) = 1.17, P = 0.25 between the two sites were insignificantly different. In the case of tomatoes, all but mean concentrations of Cr between the two sites; (Mean = (15.34 ± 13.30) mg/kg) and (Mean = ((17.97 ± 12.55) mg/kg)); t (70) = –0.63, P = 0.53, were significantly different.

Statistical analysis of levels of the studied metals in green peppers and tomatoes at the Glen Valley farms using paired sample *t*–tests showed significant differences in levels of Cd, Cu and Ni in green peppers and in tomatoes. The elevated concentration levels observed in green peppers could be influenced by the different absorption capacities of the metals by the vegetables.

When the present concentrations were compared with the safe limits given by the WHO/FAO [17], Cr, Pb and Zn were above the recommended safe limits of 2.3, 0.3 and 60 mg/kg, respectively. On the other hand, Cd and Cu were found to be within the acceptable limits of 0.2 mg/kg and 40 mg/kg, respectively. Aforementioned comparisons for Ni could not be made in this study due to absence of Ni safe limits data.

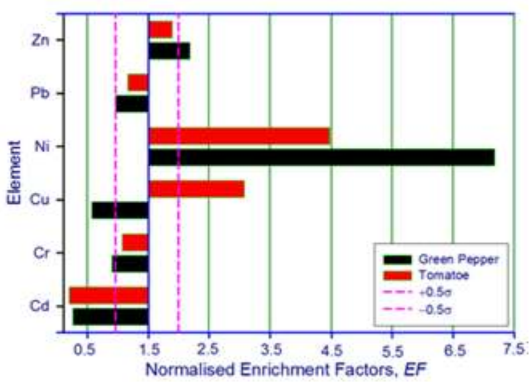

Fig. 2 Normalised enrichment of metals in wastewater irrigated vegetables

Enrichment of metals in wastewater irrigated vegetables were described in (2) and are presented in Fig. 2. It is evident that the *EF* values in tomatoes were in the order of Ni > Cu > Zn. Similarly for green peppers, the *EF* values were in the order of Ni > Zn. These results suggest that there was uptake of metals from the soils to the edible parts of the vegetables. Uptake of Pb, Cr, Cd and Cu (only in green pepper) was not observed in this study. As pointed out earlier though, mobility of metals from soil to plant is a function of the physical and chemical properties of the soil and of vegetable species, and is altered by innumerable environmental and human factors. Thus, variation of the *EF* values with different metals and different plant varieties was not unexpected.

B Cluster Analysis

Similarities among mobility of metals from soil to plant were investigated using cluster analysis which agglomerated 6 metals into three groups. The results obtained by cluster analysis are presented by dendrogram where the horizontal axis represents the degree of association between groups of variables, that is, the lower the value on the axis the more the significant the association (Fig. 3).

Dendrogram using Single Linkages
Rescaled Distance Cluster Combine

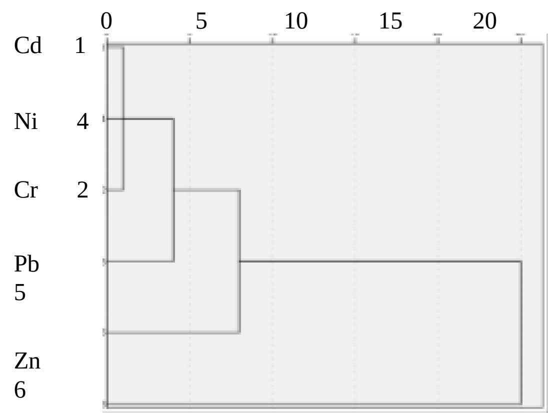

Fig. 3 Groupings of metals on the basis of translocation of metals from soils to plants by cluster analysis

As can be seen, Cd Cr, Ni and Pb are grouped into one cluster Cu and Zn are isolated. The effects and bioavailability of heavy metals depends on factors such as environmental conditions, soil pH, chemical fertilisers, genetic and cytological make up of individual plant species as well as its physiological conditions [18].

This study however, did not take the above mentioned variables into account and thus makes it difficult to draw conclusive discussions based on translocation factor categories depicted in Fig. 3.

C Daily Intake of Metals (DIM)

The daily intake of metals were estimated according to the average vegetable consumption grown in treated wastewater irrigated soils for adults and are given in TABLE III.

TABLE III
THE DAILY INTAKE (*DIM*, MG/DAY) OF CD, CR, CU, NI, PB AND ZN IN INDIVIDUAL VEGETABLES FROM GLEN VALLEY FARMS

		Cd	Cr	Cu	Ni	Pb	Zn
DIM	G/Pepper	0.09± 0.08	5.61± 4.22	16.28± 33.32	2.33± 1.13	5.02± 1.38	28.18± 11.16
	Tomato	0.07± 0.05	5.29± 4.59	2.72± 2.42	0.92± 0.63	4.72± 1.50	27.17± 14.62
	Mean	**0.08± 0.05**	**5.45± 3.12**	**9.50± 16.70**	**1.62± 0.65**	**4.87± 1.02**	**27.67± 9.20**
WHO/FAO	(mg/day)	**0.060**	**0.05–0.2**	**3**	**1.4**	**0.214**	**60**

WHO/FAO values in mg/day are based on a 60 kg body weight adult.

In the present study, the mean *DIM* values for Cr and Pb were higher than the WHO/FAO [17] values. The *DIM* values for Cd, Cu and Ni are within the same range of the Joint WHO/FAO values, considering their large standard deviations of ±0.05, ±16.70, and ±0.65, respectively. The *DIM* value for Zn (27.67 ± 9.20) mg/day is far below the 60 mg/day set by WHO/FAO [17]. According to findings of this study, it can therefore be concluded that the local population certainly ingest high levels of Cr and Pb. Once these metals enter the human body, they can lead to high health risks [19].

D Health Risk Index (HRI)

In order to assess health risk of heavy metals via dietary intake of vegetables irrigated with treated wastewater at the study area, it is useful to estimate the level of exposure to humans in each studied element to oral reference doe values, *RfD*, which are a reference point from which to gauge the potential effects of the chemical at other doses. In this present study, the vegetables were produced and sold to local supermarkets, street hawkers and individuals for consumption, and therefore the average daily intake of metals were used in calculation of the health risk, *HRI*.

The *HRI* for selected heavy metals through consumption of green peppers and tomatoes were calculated and are plotted in Fig. 4.

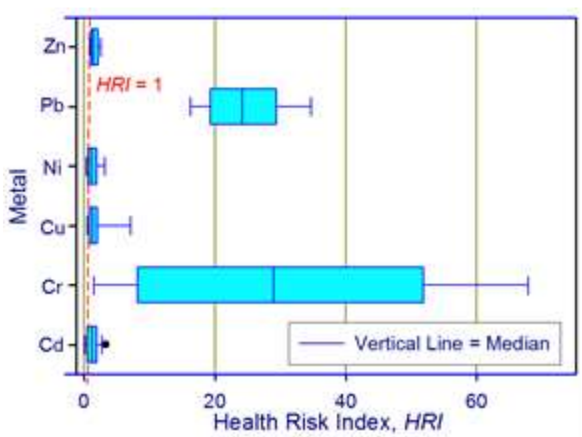

Fig. 4 Health risk index for individual heavy metals caused by consumption of green peppers and tomatoes from wastewater irrigated Glen valley farms

The *HRI* for Cd, Cr, Cu, Ni, Pb and Zn were 1.4 ± 1.2, 32.5 ± 26.1, 4.2 ± 10.9, 1.5 ± 10, 25.0 ± 7.4 and 1.7 ± 0.7, respectively. The *HRI* were for Cr and Pb from this study were far greater than 1 (*HRI* >> 1). Thus, consumption of green peppers and tomatoes from the Glen Valley farms have are of risk to the local population.

Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of Pb without visible changes in their appearance or yield [20]. Although data is limited for effects of Cr in humans, results from this study show that its *DIM* values were greater than the *RfD* values as per the US-EPA IRIS [15] and thus the *HRI* exceeds the safe value of 1. This suggests that the health risks of Cr through consumption of the studied vegetables is of great concern.

IV. CONCLUSION

The present study revealed that concentrations of Cd, Ni, Pb and Zn (in green peppers), and Cd, Cu, Ni, Pd and Zn (in tomatoes) were significantly high in treated wastewater irrigated vegetables than the ground water irrigated vegetables studied from the Glen Valley and the Ramotswa farms, respectively. When the present concentrations were compared with the safe limits, Cr, Pb and Zn were significantly higher than their respective recommended safe limits of 2.3, 0.3 and 60 mg/kg, whereas Cd and Cu were found to be within the acceptable limits of 0.2 mg/kg and 40 mg/kg, respectively. Furthermore, the daily intake values for Cr and Pb were higher than the recommended values prescribed by the Joint WHO/FAO Food Standards Program Code Alimentarius Commission whereas the *DIM* values for Cd, Cu and Ni were within the safe limits. The *DIM* value for Zn was far below the recommended safe limit. In this study, the health risks of Cr and Pb suggests high levels of exposure through consumption of the studied vegetables and was of great concern. This study is preliminary in nature, thus further monitoring is recommended considering that human health is directly affected by ingestion of heavy metals through consumption of vegetables.

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