

Human Health Risk Assessment of Heavy Metal Contamination for Population via Consumption of Selected Vegetables and Tubers Grown in Farmlands in Rivers State, South-South Nigeria

Abstract

Heavy metals contamination in farmland soils is a major concern around the world due to the potential health risk involved. The Alakahia and Eleme communities have experienced increased anthropogenic pressures due to the presence of several industries, some of which are in close proximity to the farmlands. This study was designed to investigate the heavy metals (Pb, Ni, Cd, Cu and Cr) concentration in 6 selected commonly consumed vegetables, tubers and farm soils at Alakahia and Eleme communities, Rivers state, Nigeria. The vegetables, tubers and soil samples were analyzed with Agilent FS240AA Atomic Absorption Spectrophotometer for heavy metals. All the plant samples collected from the two study sites contained detectable levels of some of the heavy metals analyzed. These metals were present in the plant samples at varying concentrations ranging from 0.01-2.78 mg/kg. Among the different metals analyzed *Dioscorea rotundata* collected from the Alakahia site however showed the highest level of Chromium (1.26 ± 0.05 mg/kg). Soil heavy metal concentrations at the sites studied were well below the maximum permissible limits. The accumulation factors differed significantly among plant species and also between both locations. The health risk index (HRI) at both study sites varied from 0.00-0.02 for Ni, 0.00-0.001 for Cr, 0.00-0.85 for Pb, 0.00-2.80 for Cd, and 0.01-0.08 for Cu. The estimated HRI for Cadmium in *Dioscorea rotundata* collected from Eleme exceeded the FAO/WHO permissible limit. However, HRI values of less than 1 indicate a relative absence of health risk associated with the ingestion of contaminated vegetables for population.

Keywords: Heavy metals; Vegetables; Risk assessment; Nigeria; Children

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Abbreviations: HRI: Health Risk Index; Pb: Lead; Ni: Nickel; Cd: Cadmium; Cu: Copper; Cr: Chromium; AAS: Absorption Spectrophotometer; AF: Accumulation Factor; DIR: Daily Intake Rate; SEM: Standard Error of the Mean; ANOVA: Analysis of Variance; EU: European Union; BW: Body Weight; TDI: Tolerable Daily Intake

Introduction

Heavy metal contamination of the soil has become a serious problem confronting both scientists and regulators around the world. The occurrence and accumulation of these heavy metals in farm soils has far reaching implications on human health as humans occupy a key position on the food chain. Within the last ten years, studies on heavy metal accumulation in food crops and agricultural soils conducted around the world have revealed a worrisome trend of heavy metal concentrations exceeding maximum permissible limits in food crops and farm soils [1-10].

Both natural and anthropogenic activities are contributing to the presence of harmful elements such as Lead, Cadmium, Mercury

and compounds such as polycyclic aromatic hydrocarbons in food crops. Examples of these activities include geologic Chibuike et al. [11] and mining activities Ma et al. [12], industrial activities Su et al. [13]. Anthropogenic agents have led to dispersion of heavy metals in the environment and consequently impaired the health of the population by consumption of victuals contaminated by harmful elements and compounds Zukowska et al. [14]. It is well known that the excessive accumulation of heavy metals can lead to a number of serious health problems and also deplete essential nutrients in humans Sohrabi et al. [15]. Furthermore, the continuous consumption of heavy metal contaminated food can result in reduced or damaged mental and central nervous system function and also damage to vital organs such as lungs, kidney and liver Raikwar et al. [16].

In Rivers state (South-South, Nigeria), the staple food diet of the populace comprises mainly of vegetables, tubers and plantains. These three food crops account for approximately 50% of the total food crops consumed National Bureau of Statistics [17]. The common vegetables consumed include Bitter leaf (*Vernonia amygdalina*), Scent leaf (*Ocimum gratissimum*), Fluted

Pumpkin (*Telfairia occidentalis*) while the root tubers include Cassava (*Manihot esculenta*), Cocoyam (*Colocasia esculenta*), Yam (*Dioscorea rotundata*) and Potato (*Ipomea batatas*).

The increasing population indices in the state has necessitated the need for the human health risk assessment posed by the heavy metals (Lead, Cadmium, Chromium, Copper and Nickel) as human health is directly affected through intake of crops grown in farm soil Oliver et al. [18]. The plant parts of interest for direct transfer of the environmental contaminants (heavy metals) to human body are the edible parts such as the leaves in vegetables and the root in root tubers which may become a threat to human health [19].

In view of this, the present study was designed and conducted to assess the potential risk of heavy metal contamination to the local population in Rivers state, South-South Nigeria via vegetables and root tubers consumption.

Materials and Methods

Study area and sampling

The study areas namely Alakahia and Eleme communities are situated in Obi-Akpor and Eleme Local Government Areas respectively. Obi-Akpor is in the metropolis of Port Harcourt, one of the major centres of economic activities in Nigeria, and one of the major cities of the Niger Delta, located in Rivers State. The Local Government Area covers 260 km² and at the 2006 Census held a population of 878,890. Eleme is located east of the Port Harcourt Local Government area. It is in the greater Port Harcourt metropolis. It covers an area of 138 km² and at the 2006 Census had a population of 190,884 Udogu [20].

Samples of commonly grown vegetables and root tubers were collected from two different sites Alakahia and Eleme both in Rivers state, Southern Nigeria. The vegetables and tubers were identified at the Plant Science and Biotechnology Department in University of Port Harcourt. For metal analysis, the edible portion of the vegetables and root tubers were used.

Soil samples were collected in November 2014. At each site i.e. the Alakahia and Eleme sites, soils at two locations were randomly sampled from the upper horizon (0-30cm). After transportation to the laboratory, soil was air-dried and sieved through a <2mm mesh and then wrapped in Aluminum foil until analysis Knoepp et al. [21].

Sample preparation

The collected vegetables and root tuber samples were washed thoroughly with deionized water to remove soil and air-borne debris. The edible parts of the samples were weighed and air-dried in a clean section of the laboratory for 4 days to reduce water content. Dried samples were then powdered using a pestle and mortar and sieved through a muslin cloth Abbasi et al. [22].

Sample digestion

For each food crop, three powdered samples from each soil site (1g each) were weighed and placed in crucibles, three replicates for each sample. The containers were then introduced into a furnace to derive ash for 8hrs (at 550°C). After 8 hours, a crucible thong was used to carry out the crucible from the furnace

into a desiccator and allowed to cool. The ash was digested with perchloric acid and nitric acid (1:4) solution Hseu (2004). The samples were left to cool and made up to a final volume of 100ml with deionized water. The hydrolyzed samples were well shaken and transferred to a centrifuge tube for centrifugation at the rate of 3000rpm to remove solid particles. The resulting homogenized samples were thoroughly mixed before subsamples were taken for metal analysis Orisakwe et al. [23]. The presence of Lead, Cadmium, Nickel, Copper and Chromium were analyzed in the sample using Agilent Atomic Absorption Spectrophotometer (AAS) FS240AA Model at 283.3nm, 228.8nm, 232.2nm, 250.5nm and 357.9nm respectively. The air-acetylene flame technique was employed for the AAS. The limit for the detection for the 5 heavy metals was all 0.001ppm with blank values reading as 0.00ppm for all the metals in deionized water. The samples were analyzed in triplicates.

Quality control

To assure the reliability of the results gotten in this present study, appropriate quality procedures were carried out. Reagents used to calibrate the instrumentation were of analytical grade. Furthermore, to assess contamination and reliability of data the coefficients of variation of replicate analysis were determined for precision of analysis; the variations were found to be less than 10%.

Data analyses

Estimation of accumulation factor: Heavy metals have the capability of translocation from soil to the edible parts of food crop that can be determined by accumulation factor (AF) Li et al. [24]. The AF's for the selected heavy metals were calculated as in the following equation:

$$\text{Accumulation Factor} = \frac{\text{Heavy metal concentration in food crop edible part}}{\text{Heavy metal concentration in soil}}$$

Estimation of daily intake rate of metals: The daily intake rate of metals (DIR) was calculated by the following equation Orisakwe et al. [23]:

$$\text{Daily Intake Rate} = \frac{C_{\text{metal}} \times D_{\text{food intake}}}{B_{\text{average weight}}}$$

Where C_{metal} is Heavy metal concentration in plants ($\mu\text{g/g}$).

$D_{\text{food intake}}$ is daily intake of vegetable (kg/person).

$B_{\text{average weight}}$ is average body weight.

The average body weights for adults and children were considered to be 55.9kg and 32.7kg respectively, while average daily vegetable intakes for adults and children were considered to be 0.345 and 0.232-kg/person/day respectively [25,26].

Estimation of health risk index: HRI refers to the ratio of the daily intake of metals in the food crops to the Oral reference dose USEPA [27] and was calculated using the following equation:

$$\text{Health Risk Index} = \frac{\text{Daily Intake Metal}}{\text{Oral reference Dose}}$$

An HRI > 1 for any metal in food crops means that the consumer population faces a health risk.

Statistical analysis: The results are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed for between and within group comparison while student's t-test was used for paired comparison. The confidence level was set at 95% (p ≤ 0.05).

Results

Heavy metals in vegetables and root tubers

Table 1 shows the Cadmium, Chromium, Lead, Nickel and Copper levels (mg/kg) in commonly consumed vegetables and root tubers in Alakahia and Eleme, Southern Nigeria. The heavy metals present in the vegetables and root tuber samples occurred at varying concentration ranging from 0.00-1.26, 0.00-0.46,

0.00-0.55, 0.11-2.78, 0.03-0.93 mg/kg for Cr, Cd, Pb, Ni and Cu respectively. The highest levels of Cr, Cd, and Cu in the vegetables and tubers were detected in *Dioscorea rotundata* (Yam) while *Vernonia amygdalina* (Bitter Leaf) and *Telfairia occidentalis* (Fluted Pumpkin) had the highest levels of Pb and Ni respectively.

Heavy metal concentrations in farm soils are shown in Table 2. The range of heavy metals in the soil samples were 0.37±0.02-0.69±0.07; 0.00±0.00-0.80±0.21; 0.01±0.00-0.03±0.00; 0.00-0.00; 0.78±0.24- 1.60±0.06; 0.52±0.05-0.90±0.17; 0.55±0.05-1.11±0.09 in Pb, Cd, As, Hg, Cr, Ni and Cu respectively. The concentrations of heavy metals (Pb, Cd and Cu) in the soil samples were significantly higher in the soil sample from Alakahia2 site when compared to the Eleme 1 & 2 sites. There is a statistically significant difference at 0.05 levels (p ≤ 0.05) among the means of the 4 sites at 95.0% confidence level.

Table 1: Heavy metals levels (mg/kg) in the commonly consumed vegetables and Tubers.

Plant/Site	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i> (A)	0.12 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.02	0.29 ± 0.02
<i>Telfairia occidentalis</i> (E)	0.17a ± 0.03	0.00 ± 0.00	0.04a ± 0.01	2.78a ± 0.29	0.81 a ± 0.22
<i>Ocimum grattissimum</i> (A)	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.02	0.16 ± 0.13	0.51 ± 0.05
<i>Ocimum grattissimum</i> (E)	0.34b ± 0.02	0.00 ± 0.00	0.26b ± 0.02	0.15 ± 0.08	0.13b ± 0.02
<i>Vernonia amygdalina</i> (A)	0.11± 0.02	0.11 ± 0.10	0.55 ± 0.05	0.20 ± 0.02	0.60 ± 0.06
<i>Vernonia amygdalina</i> (E)	0.15c ± 0.03	0.00c ± 0.00	0.41c ± 0.06	0.11c ± 0.02	0.26c ± 0.80
<i>Talinum fruticosum</i> (A)	0.03 ± 0.01	0.00 ± 0.00	0.07 ± 1.53	0.19 ± 0.03	0.30 ± 0.02
<i>Talinum fruticosum</i> (E)	0.07d ± 0.20	0.00d ± 0.00	0.10d± 0.53	0.21 ± 0.16	0.10d ± 0.01
<i>Manihot esculenta</i> (A)	0.04 ± 0.51	0.00 ± 0.00	0.21 ± 0.33	0.19 ± 0.03	0.06 ± 0.87
<i>Manihot esculenta</i> (E)	0.13e ± 0.02	0.00 ± 0.00	0.00e ± 0.00	0.13e ± 0.78	0.06 ± 0.47
<i>Colocasia esculenta</i> (A)	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.02	0.16 ± 3.27	0.51 ± 2.98
<i>Colocasia esculenta</i> (E)	0.13f ± 0.09	0.00f ± 0.00	0.10f ± 0.37	0.69f ± 0.07	0.03f ± 1.54
<i>Dioscorea rotundata</i> (A)	1.26 ± 0.05	0.00 ± 0.00	0.33 ± 0.90	0.70 ± 0.23	0.93 ± 0.18
<i>Dioscorea rotundata</i> (E)	0.11g ± 0.02	0.46g ± 0.06	0.17g ± 0.03	0.21g ± 0.02	0.23g ± 0.02

Alphabets (A,B,C,D,E,F,G) denotes significant difference when metal values for each plant from both sites are compared.

(A) Means plant from Alakahia. (E) Means plant from Eleme.

Table 2: Concentration of Heavy Metals in Soil Samples from Alakahia 1, Alakahia 2, Eleme 1 and Eleme 2.

Site	Pb	Cd	As	Hg	Cr	Ni	Cu
Alakahia 1	0.37 ^{bcd} ±0.02	0.00 ^b ±0.001	0.00±0.00	0.00±0.00	0.78 ^{bcd} ±0.24	0.52 ^{bcd} ±0.05	0.55 ^{bcd} ± 0.05
Alakahia 2	0.69 ^{acd} ±0.07	0.80 ^{acd} ±0.21	0.00±0.00	0.00±0.00	1.58 ^{acd} ± 0.06	0.85 ^{acd} ±0.23	1.11 ^{acd} ± 0.09
Eleme 1	0.41 ^{abd} ±0.04	0.00 ^b ± 0.00	0.03±0.00	0.00±0.00	1.60 ^{ad} ± 0.06	0.90 ^{abd} ±0.17	0.87 ^{abd} ± 0.23
Eleme 2	0.43 ^{abc} ±0.04	0.00 ^b ± 0.00	0.01±0.00	0.00±0.00	1.44 ^{abc} ± 0.08	0.63 ^{abc} ±0.06	0.64 ^{abc} ± 0.06

Superscript (a) shows significant difference when compared to Alakahia 1.

Superscript (b) shows significant difference when compared to Alakahia 2.

Superscript (c) shows significant difference when compared to Eleme 1.

Superscript (d) shows significant difference when compared to Eleme 2.

Table 3.1 shows the accumulation Factor (on dry weight basis) for vegetables and tuber samples grown in Alakahia, Rivers state, Southern Nigeria. For the green leafy vegetables, *Vernonia amygdalina* had the highest accumulation factor for Cr and Pb while *Ocimum grattissimum* had the highest accumulation factor for Ni and Cu. Similarly, among the three tubers studied, *Dioscorea rotundata* (yam) had the highest accumulation factor for Cr, Pb, Ni and Cu. The trend observed for accumulation factor for heavy metals in the vegetable samples are as follows; Cu>Ni>Cr>Pb>Cd; Cu>Ni>Pb>Cr>Cd; Pb>Cu>Ni>Cr>C; for *T. occidentalis*, *O. grattissimum* and *V. amygdalina* respectively. The following order was also observed for heavy metals accumulation in the tuber samples collected from the Alakahia site; Pb>Cu>Ni>Cr>Cd; Pb>Ni>Cu>Cr>Cd; Ni>Cu>Pb>Cr>Cd for *C. esculenta*, *M. esculenta*, *D. rotundata* respectively.

Table 3.1: Accumulation Factor (on dry weight basis) for vegetables and root tubers grown in Alakahia.

Vegetable/ Root Tuber	Cr	Pb	Cd	Ni	Cu
<i>Telfairia occidentalis</i>	0.1	0	0	0.17	0.34
<i>Ocimum grattissimum</i>	0	1.16	0	1.33	3
<i>Vernonia amygdalina</i>	0.14	1.49	0	0.38	1.07
<i>Talinum fruticosum</i>	0.02	0.19	0	0.24	0.18
<i>Manihot esculenta</i>	0	1.03	0	0.3	0.93
<i>Colocasia esculenta</i>	0.03	0.3	0	0.22	0.05
<i>Dioscorea rotundata</i>	0.86	0.89	0	0.96	0.94

Table 3.2 shows the variation in accumulation factors for vegetables and root tuber samples collected from the Eleme site. The trend observed accumulation factor for heavy metals in the green leafy vegetables are in the following order: Ni>Cu>Cr>Pb>Cd; Pb>Cr>Ni>Cu>Cd; Pb>Cu>Ni>Cr>Cd for *T. occidentalis*, *O. grattissimum* and *V. amygdalina* respectively. Similarly, the trend for heavy metal accumulation factor in tuber samples are in the following order: Ni>Cr>Cu>Pb>Cd; Ni>Cr>Cu>Pb>Cd; Ni>Cu>Cr>Pb>Cd for *C. esculenta*, *M. esculenta*, *D. rotundata* respectively.

Table 3.2: Accumulation Factor (on dry weight basis) for vegetables and root tubers grown in Eleme.

Vegetable/ Root Tuber	Cr	Pb	Cd	Ni	Cu
<i>Telfairia occidentalis</i>	0.12	0.09	0	4.41	1.27
<i>Ocimum grattissimum</i>	0.21	0.63	0	0.17	0.15
<i>Vernonia amygdalina</i>	0.1	0.95	0	0.17	0.41
<i>Talinum fruticosum</i>	0.05	0.23	0	0.33	0.16
<i>Manihot esculenta</i>	0.09	0.02	0	1.1	0.05
<i>Colocasia esculenta</i>	0.08	0	0	0.14	0.06
<i>Dioscorea rotundata</i>	0.12	0	0	1.4	0.31

Tables 4.1 & 4.2 show the daily intake rate of heavy metals for adult and children population via consumption of vegetables and tubers grown in Alakahia, Rivers state Southern Nigeria. In Table 4.1, trends of the estimated DIR (for adult) of Chromium, Cadmium, Lead, Nickel and Copper for green leafy vegetables and tubers were in the following order; Cu>Ni>Cr>Pb>Cd; Cu>Pb>Ni>Cr>Cd; Cu>Pb>Ni>Cr>Cd; Cu>Ni>Pb>Cr>Cd; Pb>Ni>Cu>Cr>Cd and Cr>Cu>Ni>Pb>Cd for *T. occidentalis*, *O. grattissimum*, *V. amygdalina*, *C. esculenta*, *M. esculenta* and *D. rotundata* respectively. Fluted pumpkin (*T. occidentalis*) and Yam (*D. rotundata*) had the highest DIR of 0.0078 for Copper and Chromium respectively. The highest estimated DIR (0.0007) for Cadmium was seen in Bitter Leaf (*V. amygdalina*) while the highest DIR for Lead (0.0034) and Nickel (0.0043) were seen Bitter leaf (*V. amygdalina*) and Yam (*D. rotundata*) respectively. In Table 4.2, Yam (*D. rotundata*) had the highest DIR of 0.0089 for Chromium. Similarly, the green leafy vegetable Bitter leaf (*V. amygdalina*) had the highest DIR of 0.0008 and 0.0039 for Cadmium and Lead respectively. The highest estimated DIR for Nickel (0.0050) and Copper (0.0060) were seen in Yam (*D. rotundata*). The trends of the estimated DIR of Chromium, Cadmium, Lead, Nickel and Copper for green leafy vegetables and tubers were in the following order; Cu>Cr=Ni>Pb=Cd; Cu>Pb>Ni>Cd=Cr; Cu>Pb>Ni>Cd=Cr; Cu>Pb>Ni>Cd=Cr; Pb>Ni>Cu>Cr>Cd and Cr>Cu>Ni>Pb>Cd for *T. occidentalis*, *O. grattissimum*, *V. amygdalina*, *C. esculenta*, *M. esculenta* and *D. rotundata* respectively.

Table 4.1: Daily Intake Rate for adults (g person⁻¹ day⁻¹) of heavy metals through consumption of vegetables and root tubers grown in Alakahia.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0007	Nil	Nil	0.0007	0.0018
<i>Ocimum grattissimum</i>	Nil	Nil	0.0022	0.001	0.0031
<i>Vernonia amygdalina</i>	0.0007	0.0007	0.0034	0.0012	0.0037
<i>Manihot esculenta</i>	0.0002	Nil	0.0013	0.0012	0.0004
<i>Colocasia esculenta</i>	Nil	Nil	0.0022	0.001	0.0031
<i>Dioscorea rotundata</i>	0.0078	Nil	0.002	0.0043	0.0053

Table 4.2: Daily Intake Rate for children (g person⁻¹ day⁻¹) of heavy metals through consumption of vegetables and root tubers grown in Alakahia.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0009	Nil	Nil	0.0009	0.0021
<i>Ocimum grattissimum</i>	Nil	Nil	0.0026	0.0011	0.0036
<i>Vernonia amygdalina</i>	0.0008	0.0008	0.0039	0.0014	0.0043
<i>Manihot esculenta</i>	0.0003	Nil	0.0015	0.0013	0.0004
<i>Colocasia esculenta</i>	Nil	Nil	0.0023	0.0011	0.0036
<i>Dioscorea rotundata</i>	0.0089	Nil	0.0023	0.005	0.006

The daily intake rate of heavy metals for adult and children population via consumption of vegetables and tubers grown in Eleme, Rivers state Southern Nigeria are shown in Tables 5.1 & 5.2. In Table 5.1, The trends of the estimated DIR of Chromium, Cadmium, Lead, Nickel and Copper for green leafy vegetables and tubers were in the following order; Ni>Cu>Cr>Pb>Cd; Cr>Pb>Ni>Cu>Cd; Pb>Cu>Cr>Ni>Cd; Ni>Cr>Cu>Pb>Cd; Ni=Cr>Cu=Cd; Cd>Cu>Ni>Pb>Cr for *T. occidentalis*, *O. grattissimum*, *V. amygdalina*, *C. esculenta*, *M. esculenta* and *D. rotundata* respectively. The highest DIR for Chromium (0.0021) was seen in *O. grattissimum* while the highest DIR for Cadmium (0.0028), Lead (0.0025) and Copper (0.0080) were seen in *D. rotundata* (*Yam*), *V. amygdalina* (*Bitter Leaf*) and *T. occidentalis* (*Fluted pumpkin*). Table 5.2 recorded highest DIR for Chromium (0.0024), Cadmium (0.0033), Lead (0.0029) and Copper (0.0057) were seen in *O. grattissimum*, *D. rotundata*, *V. amygdalina* and *T. occidentalis*. The trends of the estimated DIR of Chromium, Cadmium, Lead, Nickel and Copper for green leafy vegetables and tubers were in the following order; Ni>Cu>Cr>Pb>Cd; Cr>Pb>Ni>Cu>Cd; Pb>Cu>Cr>Ni>Cd; Ni>Cr>Cu>Pb>Cd; Cr=Ni>Cu>Pb=Cd; Cd>Cu>Ni>Pb>Cr for *T. occidentalis*, *O. grattissimum*, *V. amygdalina*, *C. esculenta*, *M. esculenta* and *D. rotundata* respectively.

Table 5.1: Daily Intake Rate for Adults (g person⁻¹ day⁻¹) of heavy metals through consumption of vegetables and root tubers grown in Eleme.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.001	Nil	0.0002	0.0171	0.005
<i>Ocimum grattissimum</i>	0.002	Nil	0.0016	0.0009	0.0008
<i>Vernonia amygdalina</i>	0.0009	Nil	0.0025	0.0007	0.0016
<i>Manihot esculenta</i>	0.0008	Nil	Nil	0.0008	0.0004
<i>Colocasia esculenta</i>	0.0008	Nil	0.0001	0.0042	0.0002
<i>Dioscorea rotundata</i>	0.0007	0.0028	0.001	0.0013	0.0014

Table 5.2: Daily Intake Rate for Children (g person⁻¹ day⁻¹) of heavy metals through consumption of vegetables and root tubers grown in Eleme.

Vegetable/ Root tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0012	Nil	0.0003	0.0197	0.0057
<i>Ocimum grattissimum</i>	0.0024	Nil	0.0018	0.0011	0.0009
<i>Vernonia amygdalina</i>	0.0011	Nil	0.0029	0.0008	0.0018
<i>Manihot esculenta</i>	0.0009	Nil	Nil	0.0009	0.0005
<i>Colocasia esculenta</i>	0.0009	Nil	0.0001	0.0049	0.0002
<i>Dioscorea rotundata</i>	0.0008	0.0033	0.0012	0.0015	0.0016

The Health Risk Index (HRI) of heavy metals for adults and children via consumption of vegetables and root tubers grown in Alakahia are shown in Table 6.1 and 6.2 respectively. For adults, the highest HRI for Lead (0.8500) and Cadmium (0.7000) were seen in *V. amygdalina* while *D. rotundata* had the highest HRI for Chromium (0.0052), Nickel (0.0031) and Copper (0.1325). The Health risk Index (for adults) of heavy metals in all the three leafy vegetables and root tubers collected from the Alakahia were all below 1. In table 6.2, The maximum HRI for Lead (0.9750) and Cadmium (0.8000) were seen in *V. amygdalina* while *D. rotundata* had the highest HRI for Chromium (0.0057), Nickel (0.0036) and Copper (0.1650). This pattern is similar to the HRI of heavy metals for adults. However, the values of the HRI of heavy metals for children are significantly higher than those obtained for the adult population at the Alakahia site. The Health risk Index (for children) of heavy metals in all the three leafy vegetables and root tubers collected from

Table 6.1: Health Risk Index (for Adults) of Heavy metals in Vegetables and Root tubers grown in Alakahia, Obi-Akpor L.G.A Rivers state. the Alakahia were all below 1.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0005	0	0	0.0005	0.045
<i>Ocimum grattissimum</i>	0	0	0.55	0.0007	0.0775
<i>Vernonia amygdalina</i>	0.0005	0.7	0.85	0.0009	0.0925
<i>Manihot esculenta</i>	0.0001	0	0.325	0.0009	0.01
<i>Colocasia esculenta</i>	0	0	0.5	0.0007	0.0775
<i>Dioscorea rotundata</i>	0.0052	0	0.5	0.0031	0.1325

Table 6.2: Health Risk Index (for Children) of Heavy metals in Vegetables and Root tubers grown in Alakahia, Obi-Akpor L.G.A Rivers state.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0006	0	0	0.0006	0.0525
<i>Ocimum grattissimum</i>	0	0	0.65	0.0008	0.09
<i>Vernonia amygdalina</i>	0.0005	0.8	0.975	0.001	0.1075
<i>Manihot esculenta</i>	0.0002	0	0.375	0.0009	0.01
<i>Colocasia esculenta</i>	0	0	0.575	0.0007	0.09
<i>Dioscorea rotundata</i>	0.0059	0	0.575	0.0036	0.165

Table 7.1 & 7.2 show the Health Risk Index (HRI) of heavy metals for adults and children via consumption of vegetables and root tubers grown in Eleme. For the adult population, Fluted pumpkin (*T. occidentalis*) had the highest HRI for Copper (0.1250) and Nickel (0.0171) among the selected green leafy vegetables and tubers. The maximum HRI for Chromium (0.0013), Cadmium (2.8000) and Lead (0.6250) were seen in *O. grattissimum*, *D.*

rotundata (Yam) and *V. amygdalina* (Bitter Leaf). The Health risk Index (for adults) of heavy metals in all the three leafy vegetables collected from the Eleme were all below 1. It is important to note that the HRI for Cadmium (2.8000) in *D. rotundata* (Yam) exceeded the acceptable value of 1 while the HRI of other heavy metals were below 1. In Table 7.2, The trend for HRI of heavy metals in the vegetables and root tubers collected from the Eleme site are in the following order: Cu>Pb>Ni>Cr>Cd; Pb>Cu>Cr>Ni>Cd; Pb>Cu>Cr>Ni>Cd; Cu>Ni=Cr>Pb=Cd; Pb>Cu>Ni>Cr>Cd; Cd>Pb>Cu>Ni>Cr *T. occidentalis*, *O. grattissimum*, *V. amygdalina*, *M. esculenta*, *C. esculenta* and *D. rotundata* respectively. In the selected green leafy vegetables and root tubers, only the health risk index (HRI) for Cadmium (3.3000) exceeded the acceptable value of 1.

Table 7.1: Health Risk Index (for Adult) of Heavy metals in Vegetables and Root tubers grown in Eleme, Eleme L.G.A Rivers state.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0007	0	0.05	0.0171	0.125
<i>Occinum grattissimum</i>	0.0013	0	0.4	0.0006	0.02
<i>Vernonia amygdalina</i>	0.0006	0	0.625	0.0005	0.04
<i>Manihot esculenta</i>	0.0005	0	0	0.0006	0.01
<i>Colocasia esculenta</i>	0.0006	0	0.025	0.003	0.005
<i>Dioscorea rotundata</i>	0.0005	2.8	0.25	0.0009	0.035

Table 7.2: Health Risk Index (for Children) of Heavy metals in Vegetables and Root tubers grown in Eleme, Eleme L.G.A Rivers state.

Vegetable/ Root Tuber	Cr	Cd	Pb	Ni	Cu
<i>Telfairia occidentalis</i>	0.0008	0	0.075	0.0141	0.1425
<i>Occinum grattissimum</i>	0.0016	0	0.45	0.0008	0.0225
<i>Vernonia amygdalina</i>	0.0007	0	0.725	0.0006	0.045
<i>Manihot esculenta</i>	0.0006	0	0	0.0006	0.0125
<i>Colocasia esculenta</i>	0.0006	0	0.025	0.0035	0.005
<i>Dioscorea rotundata</i>	0.0005	3.3	0.3	0.0011	0.04

Discussion

Metal contamination in vegetables and tubers

Vegetables and root tubers account for approximately 50% of the food consumed in Rivers state, Nigeria National Bureau of Statistics [17]. These vegetables and tubers are an important source of energy and vital nutrients in human diet CDCP [28]. However the intake of metal contaminated vegetables and tubers may pose a risk to human health. Agricultural activities have been identified as contributors to increasing toxic metal contamination through application of various kinds of fertilizers and pesticides

[29]. All the plant samples collected from the two study sites contained detectable levels of some of the heavy metals (Pb, Cr, Cu, Ni and Cd) analyzed. These metals were present in the plant samples at varying concentrations. Among the different metals analyzed, Cd and Cr are classified as chemical hazards and maximum residual amount have been prescribed for human [30-33]. These two heavy metals were among the least occurring metals in the plant samples. Yam (*D. rotundata*) collected from the Alakahia site however showed the highest level of Chromium (1.26±0.05). Chromium and Cadmium concentrations ranged from 0.00±0.00 to 1.26±0.05 mg/kg and 0.00±0.00 to 0.46±0.03 mg/kg respectively. Exposure to heavy metals such as cadmium and chromium is of immediate environmental concern. The maximum permissible concentration of Chromium and Cadmium are 1.00 and 0.20 mg/kg respectively as reported by European Union (EU) [32]. This shows that with the exception of *D. rotundata* (Yam) collected from Alakahia and Eleme, all other plant samples were well below the maximum permissible concentration for Cadmium and Chromium. Chromium toxicity has been attributed to its highly oxidative properties while Cadmium toxicity induces tissue injury through creating oxidative stress, epigenetic changes in DNA expression, inhibition or up regulation of transport pathways particularly in the proximal segment of the kidney tubule Duffresne et al. [34].

Some of the plant samples (*O. grattissimum*, *V. amygdalina* and *C. esculenta*) collected from Alakahia exceeded the maximum permissible limit for Lead (0.30 mg/kg) in vegetables as reported by European Union [32]. Similarly, *V. amygdalina* collected from the Eleme site also exceeded maximum permissible concentration for Lead. The occurrence of Lead in food crops has become a global environmental challenge as studies conducted around the world reveal a high occurrence of Lead in commonly consumed food [2,9,35-37]. Chronic lead poisoning is characterized by neurological defects, renal tubular dysfunction and anemia. Damage of Central Nervous System is a marked feature especially in children Underwood [38] and WHO [39]. In men, lead affects the male gametes resulting in sperm abnormalities and decreased sexual desire as well as sterility Needleman et al. [40]. In women, lead poisoning is associated with abnormal ovarian cycles and menstrual disorders in addition to spontaneous abortion Needleman et al. [41]. Lead toxicity has also been associated with inhibited heme synthesis Harmon et al. [42].

In this present study, it was observed that when the heavy metal concentrations in the selected leafy vegetables and tubers (*T. occidentalis* [TO], *O. grattissimum* [OG], *V. amygdalina* [VA], *M. esculenta* [ME], *C. esculenta* [CE], *D. rotundata* [DR]) collected from the Alakahia site was compared to the Eleme site there was significant difference at $p > 0.05$. The trends of total heavy metal concentrations observed in the leafy vegetables and tubers collected from the Alakahia site were in the following order; *D. rotundata*> *V. amygdalina*> *C. esculenta*= *O. grattissimum*> *T. occidentalis*> *M. esculenta* while the following trend; *T. occidentalis* > *D. rotundata*> *C. esculenta*> *V. amygdalina*> *O. grattissimum* > *M. esculenta* was observed at the Eleme site. The observed trend does not totally conform with trends reported in previous studies conducted by Stalikas et al. [43] and Li et al. [44] where leafy vegetables have been shown to accumulate more heavy

metals than the tubers. Leafy vegetables have been credited with higher metal accumulation rate. This is due to the fact that they have higher translocation, transpiration and also fast growth rates Itanna [45]; Muchuweti et al. [36]. Leafy vegetables are also susceptible to physical contamination by soil dust and splash because of their high foliar surface areas. The results obtained in this study suggest that in addition to the crop's physiological properties, the mobility and consequently accumulation of metals in the crop can be a function of the soil's physiological properties thus accounting for varying concentrations of metals in the studied plants. The characteristic anthropogenic pressure where each farmland is situated can also be a contributing factor to the observed variation in heavy metal concentration.

Soil contamination

Heavy metals present an environmental risk in agricultural lands as metal uptake by food crops depend upon soil physicochemical properties and plant species. Results in Table 2 revealed that with the exception of Cadmium concentration at Alakahia 2, the soil heavy metal concentrations at the farmlands studied were well below the maximum permissible limits for heavy metals in soil as specified by National Environmental Protection Agency of China, NEPAC [46] and State Environmental Protection Agency SEPA [47]. The concentration of Cadmium (0.80 ± 0.21 mg/kg) at Alakahia 2 (farmland) revealed a moderate contamination threat as it exceeded the NEPAC, 1995 and SEPA, 2005 limit of 0.30 and 0.60 mg/kg respectively. This farmland is within the vicinity of a university teaching hospital and there is usually high vehicular emission around the area. Thus, it warrants a pollution warning.

Metal transfer from soil to vegetables & tubers

Principally, the food chain (soil-plant-human) pathway is recognized as one of the major pathways for human exposure to soil contamination. Soil to plant transfer is one of the key components of human exposure to metals through the food chain Li et al. [44]. The results from Table 3.1 & 3.2 showed that the accumulation factors differed significantly among plant species and also between both locations. The differences in accumulation factors may be related to soil nutrient management and soil properties Li et al. [44]. The major factor governing metal availability to plants in soils is the solubility of the metal associated with the solid phase, since in order for root uptake to occur, a soluble species must exist adjacent to the root membrane for some finite period. The rate of release and form of this soluble species will have a strong influence on the rate and extent of uptake and, perhaps, mobility and toxicity in the plant and consuming animals. The factors influencing solubility and form of available metal species in soil vary widely geographically and include the concentration and chemical form of the element entering soil, soil properties (endogenous metal concentration, mineralogy, particle size distribution), and soil processes (e.g., mineral weathering, microbial activity), as these influence the kinetics of sorption reactions, metal concentration in solution and the form of soluble and insoluble chemical species Cataldo et al. [48].

High accumulation factors in the leafy vegetables and tubers were recorded for Copper, Nickel and in some cases Lead. This

trend of copper and nickel accumulation has also been reported for vegetables by Khan et al. [3]. It was also observed that the accumulation factors decreased with increasing concentration of metals in the soil. Thus, suggesting the inverse relationship between accumulation factor and soil metal concentration. Such inverse relationship has also been reported by Wang et al. [49], in vegetables.

Daily intake rate (DIR) of heavy metals via consumption of vegetables and tubers

The estimated dietary intake of heavy metals is a vital tool required to assess the risk posed by heavy metals in food crops. The daily intake rate of heavy metals was evaluated according to the average concentration of each heavy metal in each food crops and the respective consumption rate Ge [25]; Wang et al. [26]. From the estimated daily intake rate of heavy metals shown in Tables 4.1, 4.2, 5.1 and 5.2 it can be deduced that the DIR for children was approximately 20% higher than for adults at both Alakahia and Eleme. Zheng et al. [50] however reported a lower DIR for children compared to the adult population. This present finding is a future health concern as the children have longer exposure duration through dietary intake of these vegetables.

Furthermore, the DIR of heavy metals (Cr, Pb, Ni, Cu and Cd) through the dietary intake of the selected leafy vegetables and tubers for local inhabitants at both Alakahia and Eleme were considerably higher than those recorded in the Daboashan mine area in China Li et al. [44]. The estimated DIR of Pb, Cu and Ni for the tubers: *M. esculenta* [ME], *C. esculenta* [CE], *D. rotundata* [DR] collected from Alakahia and Eleme were slightly lower than DIR of Pb, Cu and Ni for ME, CE and DR reported in Owerri, South-East Nigeria Orisakwe et al. [23].

The degree of toxicity of heavy metals to human being depends upon their daily intake. Heavy metals intake through consumption of selected vegetables in Alakahia and Eleme, Rivers state showed large variations ranging from nil (0.000) to 0.0197 g/person/day. The Joint FAO/WHO Expert Committee on Food Additives JECFA [51] has established reference values of 150.00, 3.60, 5.00, 1.00, 500.00 $\mu\text{g}/\text{kg}$ body weight (bw) for provisional tolerable daily intake (TDI) of Cr, Pb, Ni, Cd and Cu respectively. Considering the average adult and child body weight to be 55.9 and 32.7 kg as estimated by Ge [25], Wang et al. [26] and Orisakwe et al. [23], the provisional tolerable daily intake of 150.00 and 3.60 mg/kg bw for Cr and Pb becomes 8.38 and 0.20 mg/person/day respectively while intake of Ni, Cd and Cu becomes 0.28, 0.10 and 27.90 mg/person/day respectively for adult population. For child population, the TDI for Cr and Pb becomes 4.91 and 0.10 mg/person/day respectively while Ni, Cd and Cu intake becomes 0.16, 0.00 and 16.30 $\text{mg}/\text{person}/\text{day}$ respectively.

Results from Tables 4.1 and 4.2, revealed that estimated daily intake rate (DIR) for Lead, Nickel and in some cases Cadmium were generally higher than the provisional tolerable daily intake specified by JECFA, [51].

The daily intake of Cadmium (0.0028) via consumption of Yam (*D. rotundata*) at Eleme was considerably higher than the 0.001mg/kg/day oral reference dose stated by FAO/WHO, CAC [52]. Cadmium is a dangerous element because it can be absorbed

via the alimentary tract; penetrate through placenta during pregnancy, and damage membranes and DNA. Once in the human body, it may remain in the metabolism from 16 to 33 years and is connected to several health problems, such as renal damages and abnormal urinary excretion of proteins. Decrease in bone calcium concentrations and increase of urinary excretion of calcium have also been attributed to exposure to Cd, eventually causing death. It also affects reproduction and endocrine systems of women WHO [53]. Vegetables may contribute to about 70 % of Cd intake by humans, varying according to the level of consumption Wagner [54].

The biggest contribution to the Daily intake rate of heavy metals came from Yam (*D. rotundata*) and Fluted Pumpkin (*T. occidentalis*) at Alakahia and Eleme respectively. Thus, perennial intake of these contaminated food crops is likely to induce adverse health effects arising largely from Cd exposure.

Health risk of children and adult population

In order to assess the health risk of any chemical pollutant, it is essential to estimate the level of exposure by quantifying the routes of exposure of a pollutant to the target organisms. There are various possible exposure pathways of pollutants to humans but the food chain is one of the most important pathways Zhu et al. [55]. The health risk index (HRI) for children and adult population via consumption of selected vegetables at Alakahia and Eleme shown in Tables 6.1, 6.2, 7.1 and 7.2 indicated that the HRI with the exception of Yam (*D. rotundata*) collected from Eleme were all below one ($HRI < 1$). Therefore, the health risks of heavy metals exposure through the food chain was of no consequences and generally assumed to be safe.

The HRI for Cd in Yam (*D. rotundata*) collected from Eleme was 3.30 and 2.80 for children and adult population respectively. These values were higher than the reference value (1) and thus indicate a significant health risk for the children and adult population through Cadmium exposure. The present results thus indicate that the heavy metal Cadmium is the major contributor to potential health risk for the population at Eleme.

Consequently, effective measures may be necessary to cure Cadmium contamination in soil and to reduce the metal's translocation from soil to edible crops in this region, and their implications for human health should be identified urgently by in-depth studies involving other routes of exposure.

Conclusion

The metal contamination state in farmlands is very vital in monitoring heavy metals health risk of populace. Plants can directly take up metals from contaminated soils and accumulate such metals. The plants harvested from the farm soils constitute the diet of the populace. Thus, the food chain (soil-plant-human) pathway is one of the major pathways for human exposure to heavy metal contamination in farm soils in Rivers state, Southern Nigeria. In the present study, the general HRI values of less than one indicates that consumption of the selected vegetables and tubers (with the exception of *D. rotundata* collected from Eleme) will not pose a significant health risk to the children and adult

populace. This study however determined that there was a potential health risk for the local inhabitants of Eleme through consumption of Cadmium contaminated Yam (*D. rotundata*). It is therefore recommended that the local populace of Eleme should not eat large quantities of these foods so as to avoid excessive accumulation of Cadmium in the body.

Contributions

EBE and MOW were involved in the research design, CJO collected and analyzed samples for the contaminants.

References

1. Samsøe-Petersen L, Larsen EH, Larsen PB, Bruun P (2002) Uptake of trace elements and PAHs by fruit and vegetables from contaminated soils. *Environ Sci Technol* 36(14): 3057-3063.
2. Arora M, Kiran B, Rani S, Rani A, Kaur B, et al. (2008) Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry* 11(4): 811-815.
3. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution* 152(3): 686-692.
4. Lăcătușu R, Lăcătușu AR (2008) Vegetable and fruits quality within heavy metals polluted areas in Romania. *Carpth J of Earth and Environmental Science* 3(2): 115-129.
5. Lăcătușu R, Cîtu G, Aston J, Lungu M, Lăcătușu AR (2009) Heavy metals soil pollution state in relation to potential future mining activities in the Roșia Montană Area. *Carpath Journal of Earth and Environmental Science* 4: 39-50.
6. Li WF, Pan MH, Chung MC, Ho CK, Chuang HY (2006) Lead exposure is associated with serum paraoxonase 1(PON1) activity and genotype. *Environ Health Perspect* 114(8): 1233-1236.
7. Secu CV, Iancu OG, Buzgar N (2008) Lead, zinc and copper in the bioaccumulative horizon of soils from Iași and the surrounding areas. *Carpath Journal of Earth and Environmental Science* 3: 131-144.
8. Singh A, Sharma RK, Agrawal M, Marshall FM (2010) Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Tropical Ecology* 51(2S): 375-387.
9. Anim-Gyampo M, Musah SZ, Boateng A (2013) Health risks of heavy metals in selected food crops cultivated in small-scale gold-mining areas in Wassa-amenfi-West district of Ghana. *Journal of Natural Sciences* 3(5): 2225-0921.
10. Zeshan Ali, Alvina GK, Riffat NM, Mehreen N, Tayyaba K, et al. (2015) Heavy Metal Built-Up in Agricultural Soils of Pakistan: Sources, Ecological Consequences, and Possible Remediation Measures. *Soil Biology* 44: 23-42.
11. Chibuike GU, Obiora SC (2014) Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. *Applied and Environmental Soil Science* 2014: 1-12.
12. Ma L, Sun J, Yang Z, Wang L (2015) Heavy metal contamination of agricultural soils affected by mining activities around the Ganxi River in Chenzhou, Southern China. *Environmental Monitoring Assessment* 187: 731.

13. Su C, Jiang L, Zhang W (2014) A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. *Environmental Skeptics and Critics* 3(2): 24-38.
14. Zukowska J, Biziuk M (2008) Methodological Evaluation of Method for Dietary Heavy Metal Intake. *J Food Sci* 73(2): R21-R29.
15. Sohrabi M, Beigmohammadi Z, Cheraghi M, Majidifar S, Jahangard A (2015) Health risks of heavy metals for Population via Consumption of greenhouse vegetables in Hamadan, Iran. *Achieves of Hygiene Sciences* 4(4): 165-171.
16. Raikwar MK, Kumar P, Singh M, Singh A (2008) Toxic Effect of Heavy Metals in Livestock Health. *Veterinary World* 1(1): 28-30.
17. NBS (2012) Consumption Pattern in Nigeria 2009/2010. National Bureau of Statistics, China.
18. Oliver MA, Gregory PJ (2015) Soil, food security and human health: a review. *European Journal Soil Science* 66(2): 257-276.
19. Alloway TP, Gathercole SE, Kirkwood H, Elliott J (2009) The cognitive and behavioural characteristics of children with low working memory. *Child Dev* 80(2): 606-621.
20. Udogu EI (2005) Nigeria in the twenty-first century: strategies for political stability and peaceful coexistence. Africa World Press, Trenton, New Jersey, USA, p. 72.
21. Knoepp JD, Vose JM, Michael JL, Reynolds BC (2012) Imidacloprid movement in soils and impacts on soil microarthropods in Southern Appalachian eastern hemlock stands. *J Environ Qual* 41(2): 469-478.
22. Abbasi AM, Shah MH, Khan MA (2014) Wild Edible Vegetables of Lesser Himalayas Plant Sciences, pp. 360.
23. Orisakwe OE, Nduka JK, Amadi CN, Dike DO, Bede O (2012) Heavy metal health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern Nigeria. *Chem Cent J* 6(1): 77.
24. Li Q, Chen Y, Fu H, Cui Z, Shi L, et al. (2012) Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary, China. *J Hazard Mater* 228: 148-154.
25. Ge KY (1992) The Status of Nutrient and Meal of Chinese in the 1990s. *Journal of Environment Pollution and Human Health* 1(1): 1-5.
26. Wang X, Sato T, Xing B, Tao S (2005) Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Sci Total Environ* 350(1-3): 28-37.
27. USEPA (2002) A review of the reference Dose and Reference concentration Processes. United States Environmental Protection Agency, USA.
28. CDCP (2015). Fruits and vegetables: Nutrition for everyone. Centers for Disease Control and Prevention. USA.
29. Khairiah J, Ding-Woei Y, Habibah J, Ahmed-Mahir R, Aminah A, et al. (2009) Concentration of heavy metals in guava plant parts and soil in the Sungai Wangi Plantation, Perak, Malaysia. *International Journal Agriculture Research* 4: 310-316.
30. FAO (1983) Food and Agriculture Organization, Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 5-100.
31. EC (2001) Commission Regulation (EC) No 466/2001 of 8 March 2001. Official Journal of European Communities.
32. <http://eur-lex.europa.eu/oj/direct-access.html>
33. FDA (2001) Fish and Fisheries Products Hazards and Controls Guidance. (3rd edn.), Centre for Food Safety and Applied Nutrition, US Food and Drug Administration, USA.
34. Dufresne J, Farnsworth R (2001) A review of latest research findings on the health promotion on the properties of tea. *Journal of Nutritional Biochemistry* 12(7): 404-421.
35. Gidlow DA (2004) Lead toxicity. *Occupational Medicine* 54(2): 76-81.
36. Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD, et al. (2006) Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture Ecosystem Environment* 112(1): 41-48.
37. Khairah J, Zalifah MK, Yin YH, Aminah A (2004) The uptake of heavy metals by fruit type vegetables grown in selected agricultural areas. *PJBS* 7(8): 1438-1442.
38. Underwood EJ (1977) Trace elements in human and animal nutrition. (4th edn), Academic Press, New York, USA.
39. WHO (2010) Childhood lead poisoning. World Health Organization, Geneva, Switzerland.
40. Needleman H, Landrigan P (1981) The health effects of low level exposure to lead. *Ann Rev Public Health* 12: 111-140.
41. Needleman H, Rabinowitz M, Leviton A (1984) The relationship between prenatal exposure to lead and congenital anomalies. *JAMA* 251: 2956-2959.
42. Harmon RE, Holm PE, Lorenz SE, McGrath SP, Christensen TH (2004) Metal uptake by plants from sludge amended soils; caution is required in the interpretations. *Plant and soil* 216(1-2): 53-64.
43. Stalikas CD, Chaidou, C.I., Pilidis, G.A. (1997). Enrichment of PAHs and heavy metals in soils in the vicinity of the lignite-fired power plants of West Macedonia (Greece). *Sci Total Environ* 204: 135-46.
44. Li Z, Zhuanga P, McBride MB, Xia H, Lia N (2009) Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of The Total Environment* 407(5): 1551-1561.
45. Itanna F (2002) Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiop J Health Dev* 16(3): 295-302.
46. NEPAC (2005) Maximum Levels of Contaminants in Food. National Environmental Protection Agency of China, Beijing, China.
47. SEPA (2005) The Limits of Pollutants in Food. State Environmental Protection Administration, China.
48. Cataldo DA, Wildung RE (1978) Soil and plant factors influencing the accumulation of heavy metals by plants. *Environ Health Perspect* 27: 149-159.
49. Wang G, Su MY, Chen YH, Lin FF, Luo D, et al. (2006) Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. *Environ Pollut* 144(1): 127-135.
50. Zheng N, Wang Q, Zhang X, Zheng D, Zhang Z, et al. (2007) Population health risk due to dietary intake of heavy metals in the industrial

- area of Huludao city, China. *Sci Total Environ* 387(1-3): 96-104.
51. JEFCA/FAO/WHO (1999) Joint FAO/WHO Expert Committee on Food Additives Toxicological Evaluation of Certain Food Additives. ILSI Press International Life Sciences Institute: Washington. USA.
 52. FAO/WHO/CAC (2013) Codex Alimentarius Commission. (21st edn), Procedural Manual, Rome Italy, pp. 106-162.
 53. WHO (2004) Evaluation of certain food additives and Contaminants. World Health Organization, Geneva, Switzerland.
 54. Wagner GJ (1993) Accumulation of cadmium in crop plants and its consequences to human health. *Advances in Agronomy* 51: 173-212.
 55. Zhu YG, Khan S, Cao S, Zheng YM, Huang YZ (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution* 152(3): 686-692.
 56. FAO/WHO (1999) Joint FAO/WHO Expert Committee on Food Additives Toxicological Evaluation of Certain Food Additives. ILSI Press International Life Sciences Institute: Washington. USA.
 57. USEPA (2010) Overview of IRIS Human Health Effect Reference and Risk Values. United States Environmental Protection Agency, Washington, USA.
 58. USEPA (2008) Contract Laboratory Program National Functional Guidelines for Superfund Organic Methods Data Review. United States Environmental Protection Agency, USA.
 59. Zeng-Yei H (2004) Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresource Technology* 95(1): 53-59.