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Evaluation of Potential Dietary Toxicity of Heavy Metals of Vegetables

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Abstract

Introduction: Consumers are exposed to a diversity of chemicals in all areas of life. Air, water, soil and food are all unavoidable components of the human environment. Each of those elements influences the quality of human life, and each of them may be contaminated.

Objective: Levels of cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) in vegetables and soils from Ohaji, Umuagwo and Owerri in southern Nigeria were determined and the potential health risks assessed.

Methods: Commonly grown vegetables, fruits and food crops were collected from three different sites in southern Nigeria, washed, oven-dried in a hot air oven at 70–80°C for 24 h. Dried samples were powdered using pestle and mortar and sieved through muslin cloth. Samples (0.5 g each) were digested with perchloric acid and nitric acid (1:4) solution. The presence of lead, cadmium and nickel were analyzed in samples using the Unicam Atomic Absorption Spectrophotometer (AAS) Model 929.

Results: Concentrations of Cd, Ni and Pb in Ohaji exceeded maximum allowable concentrations for agricultural soil. Cadmium, Ni, and Pb in vegetables were highest in *Murraya koenigii*, *Piper guineense* and *Amaranthus viridis* Linn, respectively. The estimated yearly intake of Pb, Cd and Ni in commonly consumed vegetables, Green leaf (*Amaranthus viridis*), fluted pumpkin (*Telfaria occidentalis*) and Curry leaf (*Murraya koenigii*) in Nigeria were calculated to be 1,210, 150 and 456 mg.kg⁻¹, respectively.

Conclusion: Taken together it might be concluded that these vegetables may contribute to the body burden of heavy metals especially lead.

Keywords: Environmental toxicology; Nigeria; Risk assessment

Introduction

Contamination of foods by heavy metals has become a challenge for producers and consumers. Air, soil, and water pollution contribute to presence of cadmium (Cd), mercury (Hg) and lead (Pb) in foods. The occurrence of heavy metals in the ecosystem is associated with rapid industrial growth, overuse of synthetic agricultural chemicals, or pollution produced by humans [1].

Vegetables contribute protein, vitamins, iron, calcium and other nutrients to the human diet [2]. Metal accumulation in vegetables may pose a threat to human health [3,4]. Heavy metals are easily accumulated in edible parts of leafy vegetables [5]. Vegetables can take up and accumulate heavy metals in quantities high enough to cause clinical problems to humans [6]. A number of serious health problems can develop as a result of excessive uptake of heavy metals. The consumption of heavy metal-contaminated food can seriously deplete essential nutrients in the body causing, or contributing to, a number of diseases [7].

The project was undertaken to quantify lead (Pb), cadmium (Cd), nickel (Ni) and mercury (Hg) concentrations in soil and vegetables commonly grown, or sold, in southeastern Nigeria to evaluate the potential dietary toxicity. The effect of transfer factors of heavy metals from soil sites were studied in vegetables to determine concentrations of accumulated metals to which humans are exposed.

Materials and Methods

Samples of vegetables were collected from three towns Ohaji, Umuagwo and Owerri in Imo State, southern Nigeria. For metal analysis, only edible parts of vegetables were used. Samples were

washed with deionized water. Edible parts of samples were weighed and air-dried for a day, to reduce water content. All samples were oven-dried at 70–80°C for 24 h. Dried samples were ground using a pestle and mortar and sieved through Muslin cloth.

For each vegetable three samples from each location (0.5 g each) were weighed and placed in crucibles for ashing; three replicates for each sample. The ash was digested with perchloric acid and nitric acid (1:4 v:v) solution. Samples were cooled and made up to a final volume of 25 mL with deionized water. Hydrolyzed samples were shaken and transferred to a tube for centrifugation at 3000 g. Samples were mixed before sub-samples were analyzed to ensure homogeneity of the mixture. Presence of Cd, Ni and Pb were analyzed using an Atomic Absorption Spectrophotometer (AAS; Model 929, Unicam, Cambridge, England).

Mercury was determined by the cold vapor technique after reduction with stannous chloride (SnCl₂). A stock standard solution was prepared by dissolving 1.08 g of mercury (II) oxide, in a minimum volume of 1:1 v:v HCL and diluted to 1 L with deionized water. This

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solution was analysed by the AAS using an air-acetylene, oxidizing (lean, blue), flame at a wavelength of 253.7 nm. The limit of detection for Cd, Hg, Ni, and Pb were all 0.001 $\mu\text{g.g}^{-1}$ with blank values reading as 0 $\mu\text{g.g}^{-1}$ for all metals in deionized water, with electrical conductivity values of lower than 5 $\mu\text{S.cm}^{-1}$. Samples were analyzed in triplicate.

Reagents used to calibrate the instrumentation were analytical grades. A spike-and-recovery analysis was performed to assess the accuracy of the analytical techniques used. Post-analyzed samples were spiked and homogenized with varying amounts of standard solutions of the different metals. Spiked samples were processed for analysis by the dry ashing method. Coefficients of variation of replicate analysis were determined for precision of analysis and were <10%. Transfer factor (TF) was calculated to understand the extent of risk and associated hazard due to ingestion consequent upon heavy metal accumulation in edible portion of vegetables:

TF = Concentration of metal in edible part/concentration of metal in soil

The daily intake rate of metals (DIR) was calculated by the following equation:

$$\text{DIR} = C_{\text{metal}} \times D_{\text{food intake}} / B_{\text{average weight}}$$

Where: C_{metal} = heavy metal concentration in plants ($\mu\text{g.g}^{-1}$)

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

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

The average adult body weight was considered to be 55.9 kg, and average daily vegetable intake for adults considered to be 0.345 kg/person/day [8,9]. In estimated or calculated levels of Cd and Pb in the food sample yearly averages as determined by Akpokodje et al. [10] and Inter-réseaux [11] were used.

Results

The highest mean levels of Cd, Hg, Ni, and Pb in the vegetables were detected in leaves of curry (*Murraya koenigii* Sprengel (L.)), black pepper (*Piper guineense*), green leaf *Amaranthus viridis* Linn) and garden egg (*Solanum melongena* Linn) (Table 1). Mercury was not detected in any soil sample. The range of various metals in soil samples was 0.00-3.53, not detected, 0.26-1.56 and 0.00-0.18 for Cd, Hg, Ni and Pb, respectively. Concentrations of Cd, Ni and Pb in Ohaji 1, 2 and 3 soil samples exceeded the maximum allowable concentrations for agricultural soil as recommended by EU but were lower than Canadian human quality health soil quality guidelines (Table 2).

The transfer factors TF of Cd, Ni and Pb to different vegetables at Ohaji and Umuagwo soil sites differed (Table 3). The TF of Pb ranged from 0.00-5.72 at Ohaji 1 soil for Curry leaf, while Cd ranged from 0.00-3.70 at Ohaji 2 soil sample for black pepper leaf and Ni was 0.00-1.46 at Umuagwo for Green leaf. The highest TF of Pb was found in Ohaji 1 soil for Curry leaf (*Murraya koenigii*). The daily intake rate (g/person/day) of Cd, Hg, Ni and Pb DIM through consumption of vegetables differed (Table 4). The estimated yearly intake in three commonly consumed vegetables Green leaf (*Amaranthus viridis*), fluted pumpkin (*Telfaria occidentalis*) and Curry leaf (*Murraya koenigii*) in Nigeria were 150, 456 and 1,210 mg.kg^{-1} for Cd, Ni and Pb, respectively (Table 5).

Discussion

Values for heavy metals were lower in soils than in plant tissues. Intake of heavy metal contaminated vegetables by humans through

| Crop | Pb | Cd | Ni | Hg |
|--|------|------|------|-----------------|
| Fluted pumpkin (<i>Telfaria occidentalis</i>) | 0.56 | 0.25 | 0.66 | Nd ^a |
| Scent leaf (<i>Ocimum gratissimum</i>) | 0.88 | 0.34 | 0.49 | Nd |
| <i>Gnetum africana</i> | Nd | 0.17 | 0.35 | Nd |
| Black pepper leaf (<i>Piper guineense</i>) | 1.38 | 0.37 | 1.27 | Nd |
| Green leaf (<i>Amaranthus viridis</i>) | 1.60 | 0.27 | 1.30 | Nd |
| Waterleaf (<i>Talinum triangulare</i>) | 0.94 | 0.12 | 0.59 | Nd |
| Bitterleaf (<i>Vernonia amygdalina</i>) | 0.35 | 0.13 | Nd | Nd |
| <i>Gangronema latifolium</i> | 1.27 | 0.31 | 0.99 | 0.02 |
| Garden egg leaf (<i>Solanum melongena</i>) | 0.80 | 0.35 | 0.67 | 0.03 |
| Curry leaf (<i>Murraya koenigii</i>) | 3.89 | 0.23 | 0.32 | Nd |
| Okra (<i>Abelmoschus esculentus</i>) | Nd | 0.11 | 0.08 | Nd |
| Cucumber (<i>Cucumis sativus</i>) | Nd | Nd | 0.80 | Nd |
| White camwood (<i>Pterocarpus mildbreadii</i>) | Nd | Nd | 1.00 | Nd |

^a Nd = not detectable.

Table 1: Lead, cadmium nickel and mercury levels (mg.kg^{-1}) in vegetables.

| Sample | Standard | Cd | Pb | Ni |
|--|--|--------------------|---------|--------|
| Soil, $\mu\text{g.g}^{-1}$ | Indian Standard Awashthi | 3-6 | 250-500 | 75-150 |
| | WHO/FAO, 2007 | - | - | - |
| | European Union, 2002 | 3-0 | 300 | 75 |
| Soil, mg.kg^{-1} | Canadian human quality health soil quality guideline | 14 | 140 | |
| Plant, $\mu\text{g.g}^{-1}$ | Indian Standard Awashthi | 1.5 | 2.5 | 1.5 |
| | WHO/FAO, 2007 | 0.2 | 5.0 | - |
| | Commission regulation, EU, 2006 | 0.2 | 0.30 | - |
| Leaf vegetables | European Union maximum levels in foods (mg.kg^{-1} wet weight) | 0.20 ^a | 0.3b | |
| Vegetables, excluding leaf vegetables | | 0.050 ^a | | |
| Stem vegetables, root vegetables, and potatoes | | 0.10 ^a | | |

^a according to Commission Regulation (EC) 1881/2006 latest amended by Regulation (EC) No 629/2008.

^b FAO/WHO (2001), Joint Codex Alimentarius Commission.

Table 2: Guideline for safe limits of heavy metals.

the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries. These health risk assessment depends on chemical composition of the soil, its physical characteristics, type of vegetables cultivated and consumption rate of crops grown on the soils [12]. Uptake of heavy metals by plants is often influenced by plant species, growth stage, soil type, metal species and environmental factors. Heavy metal concentrations in the soil solution play a critical role in controlling metal availability to plants. Increasing levels of heavy metals in the soil may cause increased uptake by plants. Availability of heavy metal ions are influenced by various factors including soil pH, physical and chemical soil properties, clay content and Mn oxide concentration [13]. Some plants are capable of taking up lead from soil through their root systems; although this uptake does not appear to be appreciable [14]. Only about 0.005 to 0.13% of lead in the soil solution is available to plants. The absorption of lead by roots is passive, and low. Levels of lead in leaves often correlate with atmospheric Pb concentrations [15]. Soil samples from land where vegetables were harvested indicated presence of Cd, Ni and Pb. Mercury was not detected in soil samples. The concentration of soil Pb was highest followed by Ni and Cd.

Results of this and previous studies [16,17] demonstrate that plants

| Vegetable | Lead | | | Cadmium | | | Nickel | | |
|--|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Ohaji 1 | Ohaji 2 | Umuagwo | Ohaji 1 | Ohaji 2 | Umuagwo | Ohaji 1 | Ohaji 2 | Umuagwo |
| Fluted pumpkin (<i>Telfaria occidetalis</i>) | 0.82 | 1.97 | Nil | 1.56 | 2.50 | 1.39 | 0.73 | 0.42 | 0.74 |
| Scent leaf (<i>Ocimum gratissimum</i>) | 1.29 | 0.25 | Nil | 2.13 | 3.40 | 1.89 | 0.54 | 0.31 | 0.55 |
| <i>Gnetum africana</i> | Nil | Nil | Nil | 1.06 | 1.70 | 0.94 | 0.39 | 0.22 | 0.39 |
| Black pepper leaf (<i>Piper guinenese</i>) | 2.03 | 0.39 | Nil | 2.31 | 3.70 | 2.05 | 1.41 | 0.81 | 1.43 |
| Green leaf (<i>Amaranthus viridis</i>) | 2.35 | 0.45 | Nil | 1.69 | 2.70 | 1.50 | 1.44 | 0.83 | 1.46 |
| Waterleaf (<i>Talinum triangulare</i>) | 1.38 | 0.27 | Nil | 0.75 | 1.20 | 0.67 | 0.66 | 0.38 | 0.66 |
| Bitterleaf (<i>Vernonia amygdalina</i>) | 0.51 | 0.10 | Nil | 0.81 | 1.30 | 0.72 | Nil | Nil | Nil |
| <i>Gangronema latifolium</i> | 1.87 | 0.36 | Nil | 1.94 | 3.10 | 1.72 | 1.10 | 0.63 | 1.11 |
| Garden egg leaf (<i>Solanum melongena</i>) | 1.18 | 0.23 | Nil | 2.19 | 3.50 | 1.94 | 0.74 | 0.43 | 0.75 |
| Curry leaf (<i>Murraya koenigii</i>) | 5.72 | 1.10 | Nil | 1.44 | 2.30 | 1.28 | 0.36 | 0.21 | 0.36 |
| Okra (<i>Abelmoschus esculentus</i>) | Nil | Nil | Nil | 0.69 | 1.10 | 0.61 | 0.09 | 0.05 | 0.09 |
| Cucumber (<i>Cucumis sativus</i>) | Nil | Nil | Nil | Nil | Nil | Nil | 0.89 | 0.51 | 0.90 |
| White camwood (<i>Pterocarpus mildbreadii</i>) | Nil | Nil | Nil | Nil | Nil | Nil | 1.11 | 0.64 | 1.12 |

Nil = zero

Table 3: Transfer factor of lead, cadmium, and nickel through different vegetables at Ohaji and Umuagwo soil sites.

grown on contaminated soils are more contaminated with heavy metals, which pose a major health concern. The levels of Cd and Ni from industrial areas were higher than those of residential areas [18].

Among possible human target organs of heavy metals, are soft tissues such as the kidney, liver and the central nervous system [19]. Some patients develop vesicular type of hand eczema following ingestion of Ni [20]. Although rare, chronic urticaria, a type 1 hypersensitivity response, has been attributed to dietary Ni [21]. The mean total dietary intake of Ni has been reported to be between 0.12-0.21 mg in the UK [22], 0.13 mg in Finland [23] 0.17 mg in the US [24] and 0.207-0.406 mg in Canada [25]. Vegetables used in Nigerian diets include green leaves, roots and tubers.

Provisional tolerable weekly intake (PTWI) depends on the amount, consumption period and contamination level of consumed food. The FAO/WHO (1993) established a provisional tolerable weekly intake (PTWI) of 25 $\mu\text{g.kg}^{-1}$ of body weight for Pb in humans, equaling 1500 μg Pb/week for a 60 kg person [26]. In 1995, the WHO estimated that total lead intake in adults' worldwide range from 105 to 2212 μg /week [26]. In Canada the dietary intake of Pb is 168 μg /week for a 60 kg person [27]. The PTWI of Cd has been set at 7 $\mu\text{g.kg}^{-1}$ body weight [28], equaling 420 μg Cd/week for a 60 kg person. The dietary intake of Cd, Ni and Pb appears to be higher in plants tested than the FAO/WHO PTWI.

The WHO/FAO [29] recommends a population dietary intake goal of more than 400 g/day for vegetables. Many advanced countries have campaigns for promoting consumption of vegetables, especially in the framework of the International Fruits and Vegetables Alliance [29,30]. In addition to the FAO-WHO initiative, this approach is supported by the Global Horticultural Initiative and is considered as a good way for reaching the United Nations Millennium Development Goals [30]. Should Nigeria adhere to this recommendation, it will be worthwhile to ascertain safety of vegetables consumed with respect to heavy metals, especially Cd and Pb. It could be that the body burden of lead in an average Nigerian exceeds that of values obtained in Europe and America, a cumulative amount from other sources may make it even higher. Dietary Pb, but not Cd might be more of a public health problem in Nigeria. Orisakwe [31] noted that while blood lead levels (BLLs) in many western countries have progressively declined over the years, in Nigeria high BLL continue to be documented in exposed and unexposed control subjects. There exist many sources of environmental Pb exposure in Nigeria, including leaded gasoline. Although there was

a plan to reduce the Pb content of Nigerian gasoline by [32] there is no evidence to suggest that the program has been implemented.

Metal contamination and infiltration profile into soil from refuse dump sites in Nigeria have been determined. Heavy metals in food samples have been found to be positively correlated with soil levels indicating soil contamination. Refuse dump sites may impact heavy metal contamination of vegetables like spinach, fluted pumpkin and cocoyam (*Xanthosoma sagittifolium* Linn).

The transfer factor provides a useful indication of relative metal availability from soils to plants [33]. Lokeshwari and Chandrappa [34] reported that cadmium is retained less strongly by the soil and is more mobile than other metals. Values for Ni and Pb are higher than for Cd and Hg due to different solubilities of these metals in soils. Metal concentrations in plants increased with increasing concentrations in surface soils. The transfer factor for mercury was zero for all soils. However, Pb had a maximum transfer factor. Variations in transfer factor among different vegetables may be attributed to differences in concentration of metals in the soil and differences in element uptake by vegetables [35,36].

The degree of toxicity of heavy metals to humans depends on the daily intake. Heavy metals intake through consumption of various types of vegetables grown and sold in southeastern Nigeria varies. The standard of FAO/WHO (1999) [37] has established a reference value for tolerable daily intake. The estimated daily intake for Cd and Pb were above tolerable daily intake rates. Body weight of humans can influence tolerance to pollutants. The DIM values for heavy metals were high when based on consumption of vegetables grown in sampled soils. Among other routes, food is one of the main sources of consumer exposure to heavy metals. Since increased dietary metals intake may contribute to development of various disorders, there is a necessity for monitoring these substances in the human diet [1]. It is recommended that people living in contaminated areas should not eat large quantities of vegetables to avoid excess accumulation of heavy metals in the body. Gidlow [38] asserted that irrespective of the pressure to reduce lead exposure in the general population and working environment, legislation must be based on genuine scientific evaluation of the available evidence. Dietary intake of food results in long-term low level body accumulation of heavy metals and the detrimental impact becomes apparent only after several years of exposure. Regular monitoring of these toxic heavy metals from effluents and sewage in vegetables is essential, to prevent excessive build-up in the food chain.

| Vegetable | Pb | Cd | Ni | Hg |
|--|--------|--------|--------|--------|
| Fluted pumpkin (<i>Telfaria occidentalis</i>) | 0.0035 | 0.0015 | 0.0041 | Nil |
| Scent leaf (<i>Ocimum gratissimum</i>) | 0.0054 | 0.0021 | 0.0030 | Nil |
| <i>Gnetum africana</i> | Nil | 0.0010 | 0.0022 | Nil |
| Black pepper leaf (<i>Piper guineense</i>) | 0.0085 | 0.0023 | 0.0078 | Nil |
| Green leaf (<i>Amaranthus viridis</i>) | 0.0099 | 0.0017 | 0.0080 | Nil |
| Waterleaf (<i>Talinum triangulare</i>) | 0.0058 | 0.0007 | 0.0036 | Nil |
| Bitterleaf (<i>Vernonia amygdalina</i>) | 0.0022 | 0.0008 | Nil | Nil |
| <i>Gangronema latifolium</i> | 0.0078 | 0.0019 | 0.0061 | 0.0001 |
| Garden egg leaf (<i>Solanum melongena</i>) | 0.0049 | 0.0022 | 0.0041 | 0.0002 |
| Curry leaf (<i>Murraya koenigii</i>) | 0.0024 | 0.0014 | 0.0020 | Nil |
| <i>Abelmoschus esculentus</i> | Nil | 0.0007 | 0.0005 | Nil |
| Cucumber (<i>Cucumis sativus</i>) | Nil | Nil | 0.005 | Nil |
| White camwood (<i>Pterocarpus mildbreadii</i>) | Nil | Nil | 0.0062 | Nil |

Nil = 0.

Table 4: Daily intake (g/person/day) of heavy metals DIM through consumption of contaminated vegetables.

| |
|---|
| True Pb intake = $(200 \text{ g} \times 1.60 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 0.56 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 3.89 \text{ mg} \cdot \text{kg}^{-1}) = 1210 \text{ mg} \cdot \text{kg}^{-1}$ |
| True Cd intake = $(200 \text{ g} \times 0.27 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 0.25 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 0.23 \text{ mg} \cdot \text{kg}^{-1}) = 150 \text{ mg} \cdot \text{kg}^{-1}$ |
| True Ni intake = $(200 \text{ g} \times 1.30 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 0.66 \text{ mg} \cdot \text{kg}^{-1}) + (200 \text{ g} \times 0.32 \text{ mg} \cdot \text{kg}^{-1}) = 456 \text{ mg} \cdot \text{kg}^{-1}$ |

^ayearly intake of vegetables (Green leaf, fluted pumpkin and curry leaf) multiplied by concentration of heavy metal contaminant in each vegetable.

Table 5: Example of calculating intake^a.

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