

## RESEARCH ARTICLE

# Lead Levels in Vegetables from Artisanal Mining Sites of Dilimi River, Bukuru and Barkin Ladi North Central Nigeria: Cancer and Non-Cancer Risk Assessment

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### Abstract

Lead (Pb) contamination of foods and especially of frequently consumed vegetables is a growing public health concern worldwide. Although levels of exposure in developed countries have declined over the past decades, the same cannot be said of developing countries. Health risk assessment has increasingly been employed to determine the potential hazard of heavy metal exposure to humans. In this study vegetable samples (tomatoes, red pepper, brown beans, lettuce, cabbage, Irish potatoes, onions, green beans and carrot), soil samples, irrigation water and sediment samples were collected from the Dilimi River, Bukuru and Barkin Ladi communities in north central Nigeria and analyzed for Pb content using atomic absorption spectroscopy. The results showed levels with ranges from 0.5 – 2.4 mg/kg (Dilimi River), 0.3 – 1.7 mg/kg (Barkin Ladi) and 1.46 – 1.89 mg/kg (Bukuru) in vegetables were largely above the maximum permissible limit recommended by WHO/FAO. The lead levels found in soil samples, which ranged from 9.19 – 36.042 mg/kg, also exceeded some safety standards. At least 75% of the calculated estimated daily intakes of Pb from different vegetable samples were also higher than the permissible tolerable daily intakes PTDI (0.0035 mg/kg day<sup>-1</sup>) of Pb in both adults and children. Target hazard quotient THQ values > 1 were also observed in children. In conclusion, there is a health risk from consumption of vegetables in these mining communities.

**Keywords:** Lead level- vegetables- risk assessment- risk assessment- public health

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### Introduction

Lead (Pb) and its contamination of vegetables is of public health concern due to the frequency and quantity of consumption (Chen et al., 2013) and also over exposure to Pb continues to pose health threat globally (Mary et al., 2004). In developing countries especially in the rural areas, consumption of vegetables is higher than meat since they contain protein, vitamins, carbohydrates, iron, calcium and other nutrients (Ivey et al, 2012; Huang et al., 2014). In the last two decades, Nigeria's modern cities have experienced an increase in industrial boom, urban migration and also increased human and vehicular activities. These activities increase environmental pollution through the release of toxic substances into the atmosphere and have resulted in sundry contaminants in agricultural soils. The pollution of soil, atmosphere and water bodies by toxic metals can be persistent and irreversible (Wang et al., 2001). The presence of toxic metals such as lead (Pb), cadmium (Cd) and mercury (Hg) in the environment is of public health concern when their concentrations are higher than the maximum permissible concentration set by WHO/FAO,

US EPA and EU in different matrices (Fakhri et al., 2015; Ahmad et al., 2009). Exposure to Pb occurs mainly via inhalation and ingestion from contaminated foods, which may pose threat to human health especially children who may suffer a high health risk (elevated Blood Lead Level) due to the bio accumulative potential (Luo et al., 2012; Hu et al., 2014). The severe toxic outcomes associated with Pb exposure mark Pb as a priority contaminant in environmental and health risk assessment studies. Natural and anthropogenic activities in urban cities which include mining, smelting, industrial emission, waste incineration, coal burning and vehicle exhaust emission are sources of Pb pollution in the environment (Charlesworth et al., 2011; Wei and Yang, 2010).

Lead poisoning is a well-known disease arising from excessive intake of the metal and can damage the nervous, skeletal, circulatory, enzymatic, endocrine, and immune systems of those exposed to it (Zhang et al., 2012). Exposure to lead among populations living in developing countries occur primarily due to economic and social vulnerability when they engage in informal activities (E-waste recycling and crude mining) that release Pb

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into the environment (Pascale et al., 2016). Unlike in developed countries, the majority of the population in Nigeria are unaware of the toxic effects of Pb exposure hence the population is at high risk. Currently there are no accurate statistics on the prevalence of Pb poisoning in Nigeria. It is however assumed that the majority of the population is exposed directly or indirectly to different sources of lead in the environment (Orisakwe, 2009).

The middle belt region of Nigeria is regarded as the food basket of the nation, with bulk of the food crops including vegetables consumed in most parts of the country cultivated in this area. Until recently there has been a lack of information on the level of heavy metal pollution and the negative health effect resulting from heavy metal exposure especially Pb in agricultural soils in this area. Thus a comprehensive health risk assessment of agricultural soils in this region is urgently needed, to assess the level of soil pollution by Pb due to mining activities. This has become necessary as environmental exposure has been linked as a major contributor to the global burden of disease (Naujokas et al., 2013). The ubiquity of lead and the persistent high blood lead level in non-occupational settings (Orisakwe 2009) suggestive of dietary exposures prompted the need to begin a risk assessment of food crops from arguably Nigeria's largest food basket: Jos Plateau. Also heavy metals contamination of agricultural soils is a threat to food safety worldwide, in view of this the present study aims to determine the level of lead contamination in agricultural soils and vegetables in Bukuru, Dilimi River and Barkin Ladi mining communities in Jos, Plateau state, Nigeria. This study has evaluated the potential health risk of lead exposure from the consumption of vegetables grown in Bukuru, Dilimi River and Barkin Ladi mining communities in Jos, Plateau state employing the estimated daily intake, hazard quotient and cancer risk.

### Sample area

The Jos Plateau lies in the middle belt of Nigeria. It is approximately 104 km from North to South, and 80 km from east to West covering an area of 8,600 km<sup>2</sup>. The Jos Plateau has steep escarpment edges with a descent of about 600 m to the surrounding plains. The Southern part of Jos Plateau is in the Benue Lowlands extending towards the River Benue flood plain. Jos Plateau is situated between latitudes 10°11'N and 8°55'N and longitude 8°21'E and 9°30'E (Figure 1). The study area (Bukuru-Jos) lies between latitudes 8° 50' N and 9° 00'N and longitude 9°45'E and 9°50'E. It is about 8km from Jos town and has total land area of 22 km<sup>2</sup>; Jos plateau is known for sundry mining(iron ore, tin, aluminum, etc) from ancient times. It has an average elevation of about 1,150 metres above sea level and the highest peak some 20 km eastwards from Jos-shere hill, rising to 1,777 metres above mean sea level (Morgan, 1979). Dilimi River is the main drainage system of Jos.

## Materials and Methods

Edible parts of vegetable samples (35 vegetables namely onions, green beans, tomatoes, cabbage, lettuce,

spinach, carrot from Dilimi, 15 vegetables namely carrot, cabbage and tomatoes from Bukuru and 30 vegetables namely Red pepper, brown beans, green beans, lettuce, irish potatoes, onions from Barkin Ladi) (Table 1). Vegetable varieties were same in all the sampling sites . agricultural soil and sediment (three samples each, 0 – 10 cm depth) and water samples were collected from Dilimi, Bukuru and Barkin Ladi (Figure 1) in the vicinity of mining sites. Rainwater runoff and ponds from the mining sites constantly drained into the farmlands sampled. All samples (full sets of matched samples), vegetables, soil, sediment, and water samples were harvested and analysed in triplicates.

### Preparation of samples

The vegetable samples were air-dried, mixed well again and sieved through 0.15 mm after mechanical grinding. Soil (50 mm depth) and sediment samples were oven dried at 105 °C for 24 h, ground in porcelain mortar and sieved through a 100 mesh (30 mm), and each sample was properly stored at 4 °C until analysis. The edible parts of all the vegetable samples were washed with tap water and rinsed in deionized water to remove soil and dust particles. The samples were air dried at room temperature followed by oven drying in a hot air oven at 65 °C for 48 h to get rid of the moisture contents. Samples were powdered by a clean electric grinder, all the dried samples were sieved (1 mm) and then stored at room temperature until chemical analysis. Water samples were preserved in pre-rinsed plastic bottles with 10% solution of HNO<sub>3</sub> and stored at 4 °C.

### Lead analyses of samples

One gram of each sample was digested with HNO<sub>3</sub> and HClO<sub>4</sub> in a 5:1 ratio at 800C until a transparent solution was obtained (Sharma et al., 2007). Digestion of water samples was carried out according to the procedure reported by US EPA (1990). Solutions of digested samples were poured through Whatman filter paper No. 42 and volume was made up to 25ml with de-ionized distilled water. Lead was determined by Elemental Flame Atomic Absorption Spectrometer (FAAS Model S4 71096). All the analyses were done in triplicates.

*Health risk assessment calculation*In order to evaluate the daily or long-term potential health risk of hazardous exposure to lead (Pb) via consumption of vegetables grown in Dilimi, Bukuru and Barkin Ladi mining communities, the Estimated Daily Dose (EDD) and Target Hazard Quotient (THQ) which are models developed by the US EPA to assess risk of exposure to contaminants were calculated. If THQ value is > 1, there could be potential health risk associated with the pollutant. On the other hand, if THQ value is < 1, then, there will be no obvious risk.

### Estimated Daily Dose (EDD)

Daily intake of Pb was calculated using the equation below;

$$EDI = \frac{C_{\text{metal}} \times IR \times EF \times ED}{B_{\text{average weight}} \times AT_n}$$

### Target Hazard Quotient (THQ)

The Target hazard quotient was calculated using the following equation;

$$THQ = EDI / RfD$$

where C is the mean concentration of Pb in vegetable, IR is the daily vegetable intake by the exposed population for adults 350g and children 220g (Song et al., 2015), EF is the exposure frequency 350 days (USEPA, 2011), ED is the exposure duration of 53 years as the average life expectancy rate for a Nigerian Adult according to world bank statistics 2014, BW is the average weight of local residents 60kg for adults and 16kg for children, AT is the average exposure time for non-carcinogens (exposure days within whole lifetime) 20,440 days = 365 x 56, and RfD is the chronic oral reference dose for Pb 0.0035 mg/kg/day (USEPA, 2003).

### Incremental Lifetime Cancer Risk (ILCR)

Incremental lifetime cancer risk is the lifetime probability of an individual developing any type of cancer due to carcinogenic daily exposure to a contaminant over a life time (Li et al., 2010). The ILCR is obtained using the Cancer Slope Factor (CSF) which evaluates the probability of an individual developing cancer from oral exposure to contaminant levels over a period of a lifetime as described by ATSDR (2010) and it is contaminant specific (Pepper et al., 2012), for Pb the Ingestion cancer slope factor is 0.0085 and it is expressed in units of (mg/kg/day).

It was calculated using the equation below;

$$R = EDI \times CSF \quad (4)$$

Where R is the probability of excess lifetime cancer (or simply risk), CSF is the carcinogenic slope factor, and EDD is the estimated daily dose of lead.

### Pollution indices

The various pollution indices (Bio-concentration Factor, Contamination factor and Geochemical Index) were calculated in the study to determine the degree of pollution in the studied soil and sediment samples.

### Bio-Concentration Factor (BCF) calculation

Soil to plant metal transfer factor was calculated as the ratio of Pb concentration in plants to Pb concentration in soils. The BCF was calculated by using the following equation as follows;

$$BCF = \frac{C_{\text{vegetable}}}{C_{\text{soil}}}$$

Where C vegetable is the total concentration of Pb in the vegetable (mg kg<sup>-1</sup> dw), and C soil is the corresponding heavy metal concentration in the soil habitat of the vegetable (mg kg<sup>-1</sup>).

### Contamination Factor (CF)

$$CF = \frac{\text{Concentration of Pb in soil/Sediment}}{\text{Background Value}}$$

This model was developed by Lacatusu in 2000 to define the pollution and contamination range of soils and sediments. CF values > 1 defines the pollution range

while CF values < 1 defines the contamination range (is given as < 0.1: very slight contamination; 0.10–0.25: slight contamination; 0.26–0.5: moderate contamination; 0.51–0.75: severe contamination; 0.76–1.00: very severe contamination; 1.1–2.0: slight pollution; 2.1–4.0: moderate pollution; 4.1–8.0: severe pollution; 8.1–16.0: very severe pollution; and > 16.0: excessive pollution.). Target background value of 85 was used in this study as proposed by the Department of Petroleum Resources (DPR 2002) as the maximum permissible level of Pb in Nigerian Soil.

### Geoaccumulation index, Igeo

Geoaccumulation (Igeo) is an index defined by Müller in 1981, to determine and define metal contamination in soil and sediments (Banat et al. 2005), by comparing current concentrations with pre-industrial levels. This is also employed in the assessment of soil contamination and can be calculated as follows

$$I_{geo} = \log_2 \left[ \frac{C_n}{1.5 B_n} \right]$$

where C<sub>n</sub> is the measured concentration of the examined metal n in the soil and B<sub>n</sub> is the geochemical background concentration or reference value of the metal n.

## Results

The mean concentrations (mg/kg) of Lead (Pb) in soil, water and sediments collected from Dilimi River, Bukuru and Barkin Ladi mining areas in Jos, North central Nigeria is shown in Table 1. The mean concentrations of Pb observed in the studied communities showed large variations and was in the order lettuce > cabbage > spinach > tomatoes > green beans > carrot > onions in Dilimi River. The levels in Bukuru decreased in the following order Tomatoes > Carrot > Cabbage while the levels in Barkin ladi was in the order Green beans > Pepper > Lettuce > Beans > Onions > Irish potatoes. The mean concentrations of Pb in agricultural soils showed large variations among the different villages with mean values of 9.18, 28.81 and 36.042 mg/kg in Dilimi River, Bukuru and Barkin ladi respectively. Similarly the heavy metals concentrations in the water samples gotten from dilimi river ranged between 0.065 – 0.12. Also the heavy metals concentration in the analyzed Lead levels from sediments ranged from 39.7 - 95.13 mg/kg.

The total estimated intake rate was calculated for both Adults and children as can be seen in Table 3, the ingestion Pb through consumption of vegetables to the daily dietary intake ranged from 0.0026 – 0.012 mg/kg day<sup>-1</sup> in Dilimi, 0.007 – 0.009 mg/kg day<sup>-1</sup> in Bukuru and 0.0019 – 0.008 mg/kg day<sup>-1</sup> for adults while that of children ranged from 0.0078 – 0.035 mg/kg day<sup>-1</sup> (Dilimi), 0.0021 – 0.0027 mg/kg day<sup>-1</sup> (Bukuru) and 0.005 – 0.025 mg/kg day<sup>-1</sup> (Barkin Ladi). The calculated Bio concentration factor (BCF) was presented in figure 1, with values ranging between 0.058 – 0.26, 0.05 – 0.06 and 0.01 – 0.04 in Dilimi, Bukuru and Barkin Ladi respectively. The calculated THQ values

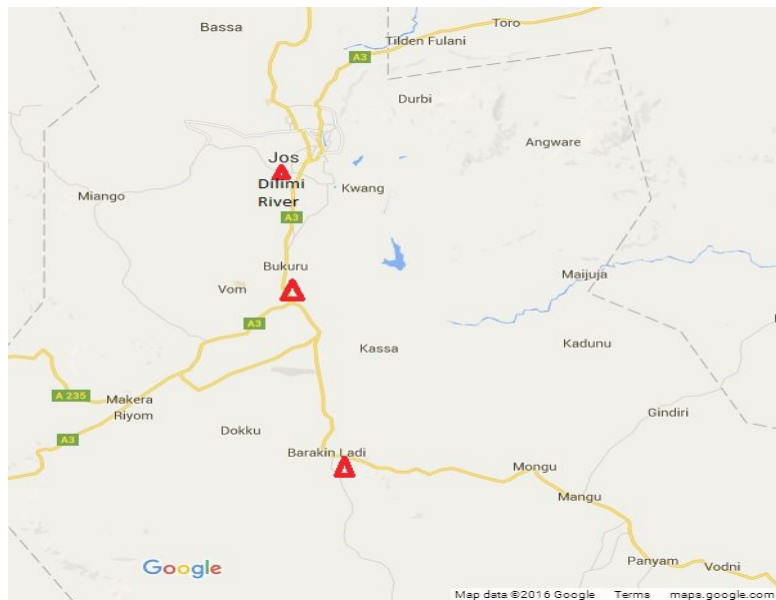


Figure 1. Location Map of Sampling Sites in the Study Area

are presented in Table 3 alongside the daily intake. THQ values for Onions, Green beans and carrot (Dilimi) and

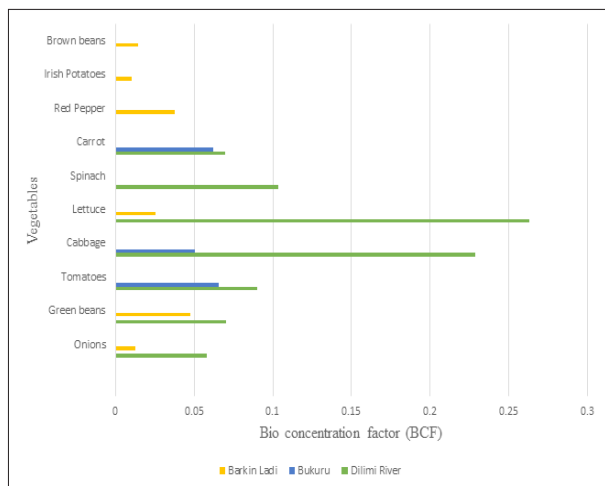


Figure 2. Bio Concentration Factor (BCF) from Soil to Vegetables in Dilimi Area

Table 1. Mean Concentrations (mg/kg) of Lead (Pb) in Soil, Water and Sediments Collected from Dilimi River, Bukuru and Barkin Ladi Mining Areas

Location Site	Pb (mg/kg)
<b>Soil Samples</b>	
Dilimi River	9.198
Bukuru	28.812
Barkin Ladi	36.042
<b>Water Samples</b>	
Dilimi River	0.0657
Bukuru	0.0994
Barkin Ladi	0.1213
<b>Sediment</b>	
Dilimi River	39.6948
Bukuru	43.9843
Barkin Ladi	95.1251

Beans, Irish potatoes and onions (Barkin Ladi) were < 1 in Adults. The THQ values in children were seen to be > 1 across the three communities.

Figure 2 is the Bio concentration factor (BCF) from soil to vegetables in Dilimi area.

### Discussion

Anthropogenic activities like mining alters the normal soil composition and is considered one of the most significant sources of lead pollution in agricultural

Table 2: Mean Levels (mg/kg) of Lead (Pb) in Vegetables Collected from Dilimi River, Bukuru and Barkin Ladi Mining Sites

Vegetables	Pb (Mg/Kg)
<b>Dilimi Rivers</b>	
Onions	0.533
Green Beans	0.646
Tomatoes	0.829
Cabbage	2.101
Lettuce	2.419
Spinach	0.953
Carrot	0.640
<b>Bukuru</b>	
Carrot	1.783
Cabbage	1.460
Tomatoes	1.889
<b>Barkin Ladi</b>	
Red Pepper	1.362
Brown Beans	0.510
Green Beans	1.723
Lettuce	0.910
Irish Potatoes	0.383
Onions	0.460

Table 3. Estimated Daily Intake (mg/kg day<sup>-1</sup> Bw<sup>-1</sup>) and Target Hazard Quotient (THQ) of Lead from Consumption of Vegetables from Dilimi, Bukuru and Barkin Ladi Areas

Vegetables	Daily Intake (mg/60kg day <sup>-1</sup> Bw <sup>-1</sup> )	THQ	Daily Intake (mg/16kg day <sup>-1</sup> Bw <sup>-1</sup> )	THQ
Dilimi River	Adults	Adults	Children	Children
Onions	0.0027	0.7619	0.0078	2.2401
Green beans	0.0032	0.9229	0.0095	2.7134
Tomatoes	0.0041	1.1845	0.0122	3.4825
Cabbage	0.0105	3.0008	0.0309	8.8222
Lettuce	0.0121	3.4565	0.0356	10.162
Spinach	0.0048	1.3613	0.014	4.0022
Carrot	0.0032	0.9148	0.0094	2.6895
Bukuru				
Carrot	0.0089	2.5469	0.0262	7.4878
Cabbage	0.0073	2.0861	0.0215	6.1332
Tomatoes	0.0094	2.6986	0.0278	7.9338
Barkin Ladi				
Red pepper	0.0068	1.945	0.02	5.7184
Brown beans	0.0026	0.7287	0.0075	2.1423
Green beans	0.0086	2.4615	0.0253	7.2369
Lettuce	0.0046	1.3011	0.0134	3.8254
Irish potatoes	0.0019	0.5473	0.0056	1.609
Onions	0.0023	0.6577	0.0068	1.9337

Table 4. Six Classes of the Geoaccumulation Index (Muller, 1981)

Class	Value	Soil quality
0	$I_{geo} \leq 0$	practically uncontaminated
1	$0 < I_{geo} < 1$	uncontaminated to moderately contaminated
2	$1 < I_{geo} < 2$	moderately contaminated
3	$2 < I_{geo} < 3$	moderately to heavily contaminated
4	$3 < I_{geo} < 4$	heavily contaminated
5	$4 < I_{geo} < 5$	heavily to extremely contaminated
6	$5 < I_{geo}$	extremely contaminated

soils (Acosta et al., 2011; Zhuang et al., 2009). The local residents living around Bukuru, Dilimi River and Barkin Ladi communities mainly engage in farming and artisanal mining as their major occupation. The three communities showed diverse concentration of Pb in agricultural soils. As shown in Table 2, the mean lead concentrations in agricultural soils collected from the three studied communities ranged from 9.198 – 36.042 mg/kg with the average mean concentration in Barkin Ladi higher than the guidance value of 20 mg/kg proposed by the Finnish government (MEF, 2007) and which have been applied in an international context for agricultural soils (UNEP, 2013). Also the concentration of Pb in soil from the three communities were lower than the Canadian and Dutch soil quality guidelines value of 600mg/kg and 530 mg/kg respectively (VROM, 2000; CCME, 2007). Although soils have heavy metal immobilizing capability, the ability of metals to bio-accumulate over a period of time will result in uptake by plants grown on contaminated soils. Since lead (Pb) is not very mobile in soil (US ASTDR, 2007),

concentrations of lead in soil which are representative of the degree of pollution/contamination may not directly be linked to an adverse health effect without conducting a health risk assessment of the plants, because only a fraction is taken up by plants. Pb is easily absorbed by soil and no toxic effect on the plants (Malakootian et al., 2009). The Pb levels in soils collected from mining communities as reported by numerous authors (Qing et al., 2015; Hu et al., 2014; Li et al., 2014; Ji et al., 2013) are higher when compared with the levels in our present study. The dissimilar trend in Pb concentration from the current study when compared with other studies, indicate that the point source of pollution differ. There are numerous sources of Pb contamination in agricultural soils which may include; fertilizers, sewage sludge, leaded gasoline from vehicular traffic and industrial emission, but recent studies have shown that mining could be a major source point of Pb pollution in agricultural soils (Hu et al., 2014).

The bioavailable metal content in soil exerts a significant impact on soil quality and this might affect food safety. Hence, the assessment of heavy metal contamination is of public health importance in agricultural soils (Loskaa et al., 2004). In this study, the assessment of the overall contamination of soil samples collected from the studied communities showed that the Igeo fell within class 0 indicating that the soil and sediment samples from the studied communities were not contaminated (Figure 2). Also assessment of sediment samples collected from Dilimi River was calculated using the contamination factor to estimate the degree of pollution. All the samples had values < 2 which indicates slight pollution.

Pb contamination of foods especially vegetables is of great concern in public health due to the frequency

of consumption among the exposed population and particularly as it affects the growth and development of children the most vulnerable population, leading to physiological and neurological disorders (Zahran et al., 2009; Khan et al., 2008; Karrari et al., 2012). These disorders are mainly as a result of increase in Blood Lead Levels (BLLs) of children living in the polluted environment (Guo et al., 2010). Ten different species of 80 vegetable samples from different locations were analyzed in this study and as shown in Table 1, with concentrations varying among different vegetable samples. The average concentration of Pb in Dilimi, Bukuru and Barkin Ladi were 1.16, 1.71 and 0.89 mg/kg respectively. These vegetable lead levels are higher than vegetables harvested from Uyo in the Niger Delta an area characterized by huge environmental pollution (Orisakwe et al., 2015). The large disparity in concentration observed in both studies could be as a result of the different contamination source. Accumulation or absorption of heavy metals by vegetables is specie dependent due to their different physiological characteristics (Pan et al., 2016; Huang et al., 2014).

The most Pb contaminated vegetables in Dilimi were tomatoes, cabbage and lettuce with mean concentration of 0.83, 2.1 and 2.42 mg/kg. In Bukuru mean concentrations of 1.78 and 1.89 mg/kg for carrot and cabbage showed the highest contamination. Also in Barkin Ladi mean levels of 1.36 and 1.72 mg/kg in pepper and green beans showed the highest concentration when compared with others. Leafy vegetables in Dilimi had higher concentrations than other vegetables as shown in Table 2, probably suggesting predominance of aerosol deposition. Vegetables lead levels in this study were similar to the reported of Jolly et al., 2013 in Bangladesh; Mahmood and Malik, 2014 in Pakistan and Harmanescu et al., 2011 in Romania. The mean levels of Pb observed in vegetables were all higher than the Maximum allowable concentration (MAC) of Pb in vegetables (FAO/WHO 2001). While blood lead levels (BLLs) in many western countries have progressively declined since 1976, the same cannot be said for Nigerians as high BLL continue to be documented not only in occupationally exposed population but also in unexposed population (Orisakwe, 2009). Lead exposure can occur through food, water, soil, air and the relative contributions from individual sources may depend on lifestyle and socioeconomic status (Othman, 2010). The increase in BLL among Nigerians may not be unconnected to environmental Pb emission from industries, mines and leaded gasoline due to limited regulatory restrictions from the relevant government agencies to check pollution.

#### Health risk assessment

In order to determine the risk posed from exposure to Pb to populations in Dilimi, Bukuru and Barkin Ladi communities via consumption of vegetables, various models proposed by USEPA to estimate the health risks of contaminants where adopted in the present study to calculate the hazards of Pb pollution/contamination. Oral intake of Pb is the most common route of Pb exposure in humans since vegetables constitute an important part of the diet (Lu et al., 2015). Therefore it is essential to estimate the level of exposure by quantifying the exposure of Pb

to the exposed population (Khan et al., 2013).

The estimated daily dose (EDD) or daily intake of Pb for both children and Adults were calculated and the values were seen to be higher in children (Table 3). Comparing the estimated daily intakes of Pb of the studied population with the probable tolerable daily intake (PTDI) of 0.0035 mg/kg day<sup>-1</sup> (JECFA 1999), 75% of samples were seen to be in violation of this standard. Although the previous PTDI was recently withdrawn by the WHO/FAO because of the difficulty in establishing a new PTDI that would be considered health protective especially to children (JECFA 2011).

The ratio of the body intake dose of Pb to the reference dose of 0.0035 (US EPA, 2003) was calculated as the THQ to estimate the potential health risk associated with long term exposure to Pb via consumption of vegetables. This shows that there will be little or no health risk associated with consumption of these vegetables grown in these areas. The vegetables with a concern level of values > 1 as shown in Figure 1 may present an adverse health outcome through a lifetime if consumption of these vegetables continues. The high THQ values observed in children ranging from >1 - >10 as shown in figure 1, is of great public health concern considering that it exceeded the critical threshold of 1. There is a need for public health awareness on the cautionary consumption of vegetables from these areas and a responsibility on the relevant government agency to remediate the agricultural soils.

#### Conflict of interest

We confirm that there is no competing interest.

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