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**ARTICLE**

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A. U. Augustine, J. C. Onwuka and C. Q. Albert

## Full Length Research Paper

# Determination of heavy metal concentration in Neem (*Azadirachta indica*) leaves, bark and soil along some major roads in Lafia, Nasarawa State Nigeria

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Soil, vegetation and atmospheric pollution have been a serious problem in recent years in Lafia, especially among the communities living along the major highways due to increase in industrialization and vehicular movements. The level of heavy metals (Cd, Cr, Ni, Pb and Zn) in Neem tree leaves, bark and soil along major roads in Lafia were determined using digestion and atomic absorption spectrophotometer methods. The leaves, trunk bark and soil samples were collected along Makurdi road (Tudun Kauri), Jos road (National Supply), Nasarawa state polytechnic site and Obi road (Maraba Akunza). The aim was to assess the level of the metals in the samples. The mean concentration of metals in the various locations along roads varied between Pb (0.028 to 0.570 mg/kg), Zn (0.061 to 1.326 mg/kg), Ni (0.028 to 0.261 mg/kg), Cr (0.013 to 0.201 mg/kg). Samples from Makurdi road (Tudun Kauri) indicated higher level of Zn, while sample from Jos road indicated the highest level of Pb. Levels of Cr and Ni in the various locations were obtained in minute quantities and were largely undetected in most of the samples. However, levels of all the metals obtained from the various locations along the major roads were below the WHO/FDA maximum permissible levels of heavy metals in plants. The Neem plant (*Azadirachta indica*) could be a good bioindicator of Pb and Zn.

**Key words:** Heavy metal, neem leaves, bark, soil, digestion, atomic absorption spectrophotometer.

## INTRODUCTION

Biomonitoring consists of the use of responses of individual plants or plant associations at several biological organization levels in order to detect or predict changes in the environment and to follow their evolution as a function of time. Some plant species are sensitive to single pollutants or to mixtures of pollutants. Those species or cultivars are likely to be used in order to

monitor the effects of air pollutants as bioindicator plants (ISEB, 2005). They have the great advantage to show clearly the effects of phytotoxic compounds present in ambient air. As such, they are ideal for demonstration purposes. However, they can also be used to monitor temporal and spatial distributions of pollution effects. Standardization of methods is crucial in order to develop

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air quality standards based on effect monitoring (ISEB, 2005).

Plants take up and accumulate elements in their tissue but can also be released back into the surrounding medium. The accumulation of an element by the plant therefore is referred to as net uptake and is based on both influx and efflux. Part of the element taken up is translocated further to other plant parts (Baker et al., 2000). The net uptake may be of different magnitude depending on the plant genotype. There are some plants called excluders that have low uptake of the element at quite high external concentrations of the element. These plants have some kind of barrier to avoid uptake, but when the external concentrations become too high, this barrier loses its function, probably due to toxic action by the element, and uptake increases massively. Other plants, called accumulators or includers, have high accumulation of elements at very low external element concentrations. These plants have certain detoxification mechanisms within the tissue, which allows the plant to accumulate such high amounts of metals. At high external concentrations, however, these plants do not increase their uptake, probably due to competition between elements at the uptake site. A special case of accumulator is the hyperaccumulator, which shows extreme accumulation of the element in leaves. For example, the concentration in leaves (mg(g dry weight)<sup>-1</sup>) is shown to be > 0.1 for Cd > 1 for Pb, Co, Cu, Ni or > 10 for Mn and Zn (Baker et al., 2000).

A heavy metal is any one of a number of elements that exhibit metallic properties, which includes transition metals, lanthanides, actinides as well as the metalloids, Arsenic and Antimony. Typically the term refers to elements of atomic number 21 or higher (for example, scandium or above). The term heavy metal chiefly arose with discussions of pollutants discharged to the environment in the form of air, water or soil contaminants. While many heavy metals have considerable toxicity, others are considered not deemed to possess significant toxic properties, and, in fact, several of these elements including zinc, iron, copper, chromium and cobalt are necessary for metabolic function for a large class of organisms. Although some heavy metals are essential micronutrients for animals, plants and many microorganisms, depending on the route and dose, all heavy metals demonstrate toxic effects on living organisms via metabolic interference and mutagenesis. Animal health impacts range from reduction in fitness, to reproductive interference to carcinoma, with many exposures being lethal (Agarwal, 2009).

Studies aimed at assessing the level of accumulation of metals and the suitability of lichens as biomonitors of any metal under interest in the area of study was carried out on epiphytic lichens on tree trunks, the host tree bark and the soil along some major roads as well as low traffic density roads in Jos to analyze the level of Cu, Zn, Pb

using atomic absorption spectrometry (AAS). The level of Cu was highest in the soil (6.22 to 10.19 µg/g) while the value in the host tree bark was 5.42 to 10.86 µg/g (Salami and Augustine, 2013).

Ahmed and Usman (2013) investigated the levels of trace metals in roadside plant and soils along some major roads in Abuja. Thirty samples, consisting of equal number of plants and soils from Airport, Kubwa and Nyanya roads were analyzed for Pb, Fe, Cu, Zn, and Cr levels using atomic absorption spectroscopy. The result of this study revealed the distribution of trace metals (Pb, Fe, Zn, Cu, and Cr) in the roadside soils and bush mint plant along some major roads in the capital city of Nigeria. Trace metal profiles decreased with distance away from Abuja roads which indicate the impact of vehicular traffic on the environment. The concentrations of trace metals in both the soils and plants are in the order of Cu > Zn > Fe > Pb > Cr in decreasing order of concentration except in the soil sample from Nyanya and the plant sample from Kubwa road where Zn is present in the highest amount. The levels of the metals are high compared to those of the control samples which indicated that there is accumulation of these metals in the soil and subsequent transfer to plants growing along the highway. Transfer factors have shown relatively higher accumulation of Pb and Fe.

Generally, the maximum levels of Pb, Fe, Zn, and Cr in soil along each road were within the safety limit guidelines proposed by most regulatory bodies. The level of copper in soil however calls for concern. Similarly, the concentration of the metals Pb, Cu and Fe in the bush mint is alarming as this was found to exceed the safety limit guidelines set by most regulatory bodies. There is significant correlation between the Pb and Fe in plant tissues and traffic volume which indicates contributions from anthropogenic activities.

Also, a study have been done to investigate trace metal concentrations (Pb, Cu, Ni, Zn, Fe) concentrations in different plants collected in the vicinity of a phosphogypsum stack in Wislinka (northern Poland). The measurements of trace metals were determined using two methods: atomic absorption spectrometry (AAS) and atomic emission spectrometry with inductively coupled plasma (OES-ICP). The mean metal contents in leaves and root of cultivated plants and weed in the Wislinka area significantly exceeded background values, while in the Osiek area; they were within the range of concentrations assumed as background. The content of trace metals in analyzed plant samples varied widely, depending on composition of soil, which was enriched by phosphogypsum particles and other environmental factors, but the detailed mechanisms are still unknown. The study shows that industrial pollution around the phosphogypsum stack in Wislinka are an important source of trace metals in crops and meadow plants. The analyzed cultivated plants from the Wislinka area are an

important source of essential trace metals and may provide larger amounts of trace metals to human body than cultivated plants from non-industrial areas (Borylo et al., 2013).

A study aimed at evaluating the differences in the bioaccumulation of some elements in *Mangifera indica* tree leaves grown around a coal fired Thermal Power Plant (affected areas) and in other areas (control) 25 km away from Thermal Power Plant (TPP) was carried out in India. The solution obtained after digestion was analyzed for Pb, Cd, Cr, Cu, Zn, Ni and Fe by AAS. The level of metals and the mode of distribution of the studied metals in mango tree grown in the periphery of TPP were not alike. In affected sites the leaves showed considerable accumulation of Pb, Cd and Cr in comparison to control areas. The seasonal changes in levels of metals of these toxic elements followed a similar pattern in affected sites with minimum values being found in monsoon and post monsoon season and maximum values in pre monsoon. This is apparently caused by the foliar deposition of fly ash on leaves during dry period, which were being washed out in pre monsoon period due to rainfall. These results also demonstrated how complicated it is to compare data from studies carried out at different times of the year (Sengupta et al., 2011).

Concentrations of some heavy metals (cobalt, cadmium, lead, nickel and chromium) in the leaves of plants viz: neem (*Azadirachta indica*), kaner (*Nerium oleander* L.), Ashok (*Saraca indica* L.) and imli (*Tamarindus indica*) around the polluted and non-polluted sites near Agra region, India was done. Heavy metal concentrations in leaves were determined by AAS. According to the findings, the order of heavy metals in plants leaves were found in as follows: Co > Pb > Ni > Cr > Cd. Correlation between heavy metals in different plants at different sites were calculated for each metal separately and a positive correlation was observed. The presence of these metal ions in plant leaves explain the fact that these plant leaves were good bioindicators and can be used in air pollution monitoring studies in industrial areas (Moh'd et al., 2012).

This present study aims at evaluating the trace metal concentration in neem leaves, trunk bark and soil along some major roads in Lafia city, Nigeria, specifically to assess the level of accumulation of Pb, Cr, Ni, Zn and Cd, to have a baseline information of the metal pollution from automobile emissions on the roadside neem plants and soil at various locations and also to suggest the continuity of the medicinal usage of the trees along the major roads which has little or no data in Lafia.

## MATERIALS AND METHODS

### Description of the study area

Lafia is the state capital of Nasarawa state and also a local

government area (LGA) in the state. It has a tropical climate with an average annual temperature and rainfall of 34.2°C and 108 mm, respectively. The highest amount of precipitation occurs in August with an average of 344.8 mm. the highest average temperature of 38°C occur between March and April. The minimum average temperature of about 19.3°C occurs in December. The variation in temperatures throughout the year is 5.9°C (Nigerian Extractive Industries Transparency Initiative (NEITI), 2013).

### Sample collection

Neem (*A. indica*) tree leaves, trunk bark and soil were collected in April, 2015 from four (4) different locations along each of these routes, Makurdi Road with high vehicular movements and commercial activities, Jos Road with great vehicular movements and commercial activities, Nasarawa state polytechnic site with high volume of automobiles movements and Obi Road with the least vehicular movements and commercial activities. Samples (leaves and bark) were collected using a stainless steel cutlass, and the soil was sampled using a sampler to a depth of about 15 to 25 cm. The samples were collected in a clean polyethylene bag each and labeled accordingly Radojevic and Bashkin (1999). The samples were transported to the laboratory for further analysis (Egereonu and Onuchukwu, 2000).

### Preparation of samples for the determination of zinc, cadmium, lead, nickel and chromium

#### Sample digestion

Wet digestion method was used and the digestion was carried out in triplicate for each sample by weighing about 1 g of the powdered sample into 100 cm<sup>3</sup> Kjeldahl flasks followed by the addition of aqua regia (HCl and HNO<sub>3</sub>) in a ratio of 3:1 (15 cm<sup>3</sup>: 5 cm<sup>3</sup>) and was left to stand for 24 h under a fume hood. The mixture was heated at 40°C for 40 min and then, the heat was increased to 100°C and the heating continued until solution became clear with disappearance of white fumes indicating the completion of the digestion process (Audu and Lawal, 2005). The digest was diluted with 10 cm<sup>3</sup> of distilled water and boiled for 15 min. The resulting solution after cooling was filtered into a 100 cm<sup>3</sup> volumetric flask using a Whatman filter paper and diluted to the mark with distilled water. This was then transferred into screw capped polyethylene bottle and stored for heavy metal determination using AAS with a digital read out system.

#### Preparation of zinc standard solution and determination

1.000 g of zinc metal was dissolved in 30 cm<sup>3</sup> of 5 M HCl solution and diluted to 1000 cm<sup>3</sup> to give 1000 ppm zinc solution. From the stock solution, 10 ppm solution was prepared by diluting 1 cm<sup>3</sup> to 100 cm<sup>3</sup>. Standards ranging from 0 to 2 ppm zinc were prepared by diluting 0 to 10 cm<sup>3</sup> of 10 ppm zinc solution to 25 cm<sup>3</sup> of separate volumetric flask. Each of the standard, the sample and also the sample blank, were aspirated at the wavelength of 213.9 nm using AAS.

#### Calculation

$$\text{Zn (\%)} = \text{Zn (ppm)} \times \text{solution volume (cm}^3\text{)}$$

$$\text{Zn (\%)} = \frac{\text{Zn (ppm)} \times \text{solution (cm}^3\text{)}}{10^4 \times \text{sample weight (g)}}$$



**Table 1.** Heavy metal concentration of the samples (mg/kg).

Sample code	Cr	Ni	Pb	Zn	Cd
N <sub>1</sub>	0.034±0.003	0.115±0.021	0.120±0.014	0.280±0.133	BDL
B <sub>1</sub>	BDL	BDL	0.075±0.010	0.085±0.019	BDL
S <sub>1</sub>	0.201±0.004	0.261±0.007	0.570±0.007	1.326±0.022	BDL

**Table 2.** Heavy metal concentration of the samples (mg/kg).

Sample code	Cr	Ni	Pb	Zn	Cd
N <sub>2</sub>	0.042±0.006	BDL	0.137±0.004	0.140±0.015	BDL
B <sub>2</sub>	BDL	BDL	0.098±0.004	0.204±0.007	BDL
S <sub>2</sub>	BDL	BDL	0.185±0.004	BDL	BDL

#### Preparation of cadmium standard solution and determination

A stock solution of cadmium was prepared by dissolving 2.0360 g of cadmium chloride in 250 cm<sup>3</sup> of deionized water and diluted to 1000 cm<sup>3</sup> in a volumetric flask, giving 1000 ppm cadmium solution. From the stock solution, the working standard (0 to 10 ppm) of cadmium solution was prepared. The standard, sample and blank solutions were aspirated at a wavelength of 229 nm and a calibration curve was prepared.

#### Calculation

Cd (ppm) = Dilution factor × graph reading

$$\text{Cd (\%)} = \frac{\text{Cd (ppm)} \times \text{solution volume (cm}^3\text{)}}{10^4 \times \text{sample weight (g)}}$$

#### Preparation of lead standard solution and determination

A stock solution of 1000 ppm lead was prepared by dissolving 1.60 g Lead (II) nitrate (Pb(NO<sub>3</sub>)<sub>2</sub>) in 20 cm<sup>3</sup> of acid and the volume made to 1000 cm<sup>3</sup> mark. 10 cm<sup>3</sup> of this solution was diluted to 100 cm<sup>3</sup> to give 100 ppm lead solution from which the working standards (0-10 ppm) was determined. Each of the standards, the sample and also the sample blank, were aspirated at a wavelength of 217.0 nm and a calibration curve was prepared.

#### Calculation

Pb (ppm) = Dilution factor × graph reading

$$\text{Pb (\%)} = \frac{\text{Pb (ppm)} \times \text{solution volume (cm}^3\text{)}}{10^4 \times \text{sample weight (g)}}$$

#### Preparation of nickel standard solution and determination

A stock solution of Nickel was prepared by dissolving 4.9530 g of Nickel nitrate (Ni(NO<sub>3</sub>)<sub>2</sub>) in 1000 cm<sup>3</sup> of deionized water and diluted to 1000 cm<sup>3</sup> in a volumetric flask giving 1000 ppm nickel solution. Each of the working standard (0-10 ppm) was prepared from the

stock solution. Each of the standards, the sample and also the sample blank were aspirated at a wavelength of 232.0 nm and a calibration curve was prepared.

#### Calculation

Ni (ppm) = Dilution factor × graph reading

$$\text{Ni (\%)} = \frac{\text{Ni (ppm)} \times \text{solution volume (cm}^3\text{)}}{10^4 \times \text{sample weight (g)}}$$

#### Preparation of chromium standard solution and determination

A stock solution of chromium was prepared by dissolving 7.6960 g of Chromium nitrate (Cr(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O) in 250 cm<sup>3</sup> of deionized water and diluted with more deionized water up to 1000 cm<sup>3</sup> mark, giving 1000 ppm chromium solution. From the stock solution the working standard (0 to 10 ppm) was prepared. Each of the standards, the sample and the sample blank were aspirated at a wavelength of 232.0 nm and a calibration curve was prepared.

#### Calculation

Cr (ppm) = Dilution factor × graph reading

$$\text{Cr (\%)} = \frac{\text{Cr (ppm)} \times \text{solution volume (cm}^3\text{)}}{10^4 \times \text{sample weight (g)}}$$

## RESULTS AND DISCUSSION

The results of the heavy metal concentration in the Neem leaves, bark and soil from the various locations are given in Tables 1 to 4. Table 1 to 4 show that N<sub>2</sub> from Jos road (National Supply) indicated the highest concentration of Pb (0.137 mg/kg), followed by N<sub>1</sub> from Makurdi road (Tudun Kauri) which had a concentration 0.120 mg/kg, then N<sub>3</sub> from Nasarawa state polytechnic site (0.056 mg/kg) and N<sub>4</sub> from Obi road (Mararaba Akunza) which

**Table 3.** Heavy metal concentration of the samples (mg/kg).

Sample code	Cr	Ni	Pb	Zn	Cd
N <sub>3</sub>	0.028±0.006	0.042±0.010	0.056±0.006	0.334±0.027	BDL
B <sub>3</sub>	BDL	BDL	0.116±0.003	0.363±0.018	BDL
S <sub>3</sub>	BDL	0.033±0.003	0.171±0.004	0.420±0.009	BDL

**Table 4.** Heavy metal concentration of the samples (mg/kg).

Sample code	Cr	Ni	Pb	Zn	Cd
N <sub>4</sub>	0.013±0.001	0.028±0.009	0.028±0.006	0.494±0.008	BDL
B <sub>4</sub>	BDL	BDL	0.140±0.004	0.316±0.020	BDL
S <sub>4</sub>	BDL	BDL	0.069±0.004	0.061±0.008	BDL

BDL - Below detection limit, N-Neem leaves, B-Bark of trunk, S-Soil, 1-Makurdi road, 2-Jos road, 3-Nasarawa state polytechnic, 4-Obi road.

had the lowest concentration of Pb (0.028 mg/kg). The high concentration of Pb in N<sub>2</sub> could probably be attributed to high commercial and automobile activities in the location which could trigger emission of fumes/smoke containing Pb (Lawal et al., 2011). The lower concentration of Pb in Neem leaves samples from other locations (N<sub>1</sub>, N<sub>3</sub> and N<sub>4</sub>) could be attributed to lower automobile and commercial activities in the areas.

Bark samples from the various locations follows the order B<sub>4</sub> > B<sub>3</sub> > B<sub>2</sub> > B<sub>1</sub>. The higher concentration of Pb in B<sub>4</sub> could be due to high automobile traffic as in the N<sub>2</sub> sample. Similar trend in the burden of heavy metals was reported in the leaves and bark of neem (*Azadirachta indica*) in katsina metropolis (Fowotade, 2005). In the soil samples, it was found that S<sub>1</sub> from Makurdi road (Tudun Kauri) had the highest concentration of Pb (0.570 mg/kg), S<sub>2</sub> and S<sub>3</sub> had lower concentrations (0.185 and 0.171 mg/kg), respectively, and was followed by S<sub>4</sub> which had the lowest concentration of Pb (0.069 mg/kg). The high concentration of Pb in S<sub>1</sub> could be due to anthropogenic activities like roadside vehicle pollution and also effluents from storage batteries in the location which contain Pb and could influence deposition of lead in the soil (Moh'd et al., 2012). It was also observed that N<sub>1</sub>, B<sub>1</sub> and S<sub>1</sub> had the highest concentration of Zn, followed by N<sub>3</sub>, B<sub>3</sub> and S<sub>3</sub>. N<sub>4</sub>, B<sub>4</sub> and S<sub>4</sub> had a lower concentration but N<sub>2</sub>, B<sub>2</sub> and S<sub>2</sub> had the lowest concentration of Zn. This result indicated that Zn is abundant in the location from which N<sub>1</sub>, B<sub>1</sub> and S<sub>1</sub> were collected. The abundance of Zn metal could be attributed to the anthropogenic activities in the vicinity of the sampled area (Blok, 2005; Oliva and Rautio, 2005). Also, it could be as result of the geology of the study area (Lawal and Batagarwa, 2005), as well as the soil topology. N<sub>2</sub>, B<sub>2</sub> and S<sub>2</sub> had the lowest concentration of Zn and could be due to lower emission, deposition and accumulation of Zn in the vicinity of the

sampled area.

It was also observed that Cr and Ni were detected only in some of the samples from the various locations while the concentration of the metals in other samples were below the detection limit and as such were not detected. S<sub>1</sub> had the highest concentration of Cr and Ni (0.201 and 0.261 mg/kg), respectively, while N<sub>4</sub> had the lowest concentration of the two metals (0.013 mg/kg for Cr and 0.028 mg/kg for Ni, respectively). The concentration of Cr was not detected in samples B<sub>1</sub>, B<sub>2</sub>, S<sub>2</sub>, B<sub>3</sub>, S<sub>3</sub>, B<sub>4</sub> and S<sub>4</sub>. Similarly, the concentration of Ni was not detected in B<sub>1</sub>, N<sub>2</sub>, B<sub>2</sub>, S<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub> and S<sub>4</sub>. This could be due to availability of these metals in amounts lower than the detection limit of the AAS used in the analysis. Largely, it could be as a result of antagonistic interactions with other elements which can reduce the uptake of these metals from both root and foliar systems (Sengupta et al., 2011). Cd was not detected in any of the samples from the various locations. This could be as a result of reduced anthropogenic activities which could lead to deposition and accumulation of Cd in the study area. Also, cadmium being a rare metal, its absorption and adsorption by plants samples are low.

The results obtained from the analysis of these metals showed that the level of concentration of all the metals analyzed in samples of Neem leaves, bark and soil from the various study areas are below the maximum tolerable limits recommended by WHO/FDA in herbal medicines. Although it is evident that the concentration of Pb in all the study areas of this research work are below the maximum tolerable level established by CODEX (WHO/FDA) of 10 mg/kg in herbal medicine, it could still be said to be on a high side. This is based on the fact that according to WHO estimates, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. Therefore, by

implication, families living along these highways under study in Lafia metropolis could be exposed to a high potential health risk associated with Pb metal as a result of use of Neem plant along these highways.

## Conclusion


The results of this study have indicated the presence of trace metals analyzed at varying degrees in samples of Neem tree leaves, trunk bark and soil, along major roads in Lafia in the order: Zn > Pb > Ni > Cr > Cd. Samples from Makurdi road (Tudun Kauri) showed highest contamination by metals, followed by samples from Nasarawa state polytechnic site with hazardous metals like Pb. Even though the levels of all metals analyzed fall below the maximum tolerable level of heavy metals in plants established by CODEX (WHO/FAD), this research work further confirms the increased danger of environmental pollution along highways due to vehicular emission.

## Conflict of interest

The authors have not declared any conflict of interest

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