Determination of heavy metal profiles in Neem leaves (*Azadirachta indica A)* along some major streets in Minna metropolis Nigeria

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Abstract

Neem (Azadirachta indica) leaves collected from some major streets in Minna metropolis were determined for their heavy metal contamination using Atomic Absorption spectrophotometer (AAS). The results showed that the concentrations of analysed heavy metals along all the sampling route ranged from 14.5±1.15- 71.0 ± 6.17 µg/g Mn; 303.0±12.23-765.0 ± 19.27 µg/g Fe; 5.8± 0.55-40.0± 3.35 µg/g Cu; 59.4±3.50-361.0±12.53 µg/g Zn, with Fe > Zn > Mn > Cu along the various routes, while Cr, Cd and Pb were below detectable limit in all the samples. The result from this study indicate that the metal ion concentration in neem leaves along the various route studied were within the permissible level as recommended by WHO for plants to be used as food or for medicinal purposes and therefore will not contribute any toxicity or harmful effect to human health when taken orally or in the form of tea or for medicinal purposes or as part of diet.

Keywords: Neem, *Azadirachta indica*, contamination, spectrophotometer, concentration toxicity

Introduction

Neem *(Azadirachta indica)* Tree is a hard fast growing evergreen tree with a straight trunk, long spreading branches and moderately thick, rough, longitudinally fissured bark. Matured trees attain a height of 7-15m (23-50feet) (Ogbuewu, 2008). The tree starts producing the yellowish ellipsoidal drupes (fruits) in about four years, and becomes fully productive in ten years and may live for more than 200years. The leaves are compound impair-pinnate, comprising up to 15 leaflets arranged in alternate pairs with terminal leaflets (Ogbuewu, 2008). The leaflets are narrow, lancelet and up to 6cm long. The flowers are abundant with sweet-smelling white panicles in the leaf axils.

The seed of Neem *(Azadirachta indica)* fruit is yellow when ripe and is about one inch long (Ogbuewu, 2008). Neem flowers mature from May to August (Koul *et al*, 2006). Neem has a strong root system with a deep tap root and extensive lateral roots. Suckers can be produced following damage to the roots (Hearne, 1975). Neem has very wide range of social economy importance. Industrially, it has been used to produce cosmetic materials like soaps and shampoos (Heukellbach *et al*., 2006) and as pesticides (Kumar, 2003).It also has its applications in medicine (Udeinya *et al*., 2008; Ganguli, 2002)

Medicinal plants are used worldwide for the treatment of several diseases and such plants also act as important sources of raw material for pharmaceutical industries that use them for the synthesis of different brand of drugs. In recent decades the use of herbal medicines has increased worldwide. This can be attributed to the fact that the side effects of these materials are often lower than synthetic drugs couple with it low cost compared to the higher costs of many conventional pharmaceutical formulations (Rates, 2000). Neem tree have been reported as one of the most important medicinal plants in different parts of the globe (Heinrich et al; 2005). In Nigeria, Neem extracts have been widely used locally for decades to treat many health problems (Sofowora, 1982). Crude extract of Neem leaves and barks are locally used for producing natural medicines and natural herbal cosmetics (Ganguli, 2002). Other workers have reported the medicinal potential of the aqueous extracts of different parts of neem plants against some pathogens (Udeinya *et al*., 2008, Chaube *et al*., 2006, Subapriya and Nagini, 2005, Baral and Chattoppadhyay, 2004, Dasgupta *et al*., 2004 and Bandyopadhyayk *et al*., 2004).

Many medical plants used in drugs formulation can present health risk due to the presence in them of some toxic substances like heavy metals. The toxicity of heavy metals depends upon the chemical form of the elements. Heavy metals are dangerous in the form of cations and are highly toxic when bonded to the short chains of carbon atoms (Hussain, 2006).
Determination of chemical composition of plants is one of the most frequently used methods of monitoring environmental pollution. Various plants have been used as bio-indicators to assess the impact of a pollution source on the vicinity which is due to high metal accumulation in the plants (Onder and Dursun, 2006). Uptake

of elements into plants can happen via roots from soil and transported to the leaves; also they may be taken up from the air, or by precipitation directly via the leaves (Kord *et al*., 2010)

Soil and vegetation pollution have been a serious problem in recent years in Nigeria, especially among the communities living along the major highways. Excessive accumulation of heavy metal in agricultural land through traffic emission may result in soil contamination and elevated heavy metal uptake by crops, and thus affect food quality and safety (Garcia and Millan, 1998).

The Nigerian situation is further exacerbated by the reality of increasing large-scale importation of old/fairly used vehicles for use on the Nigerian highways (Alo, 2008). Heavy metals are important group of pollutants. They are non-biodegradable, hence are not readily detoxified and removed by metabolic activities once they are available in the environment. This may subsequently lead to their build up to toxic levels or bioaccumulation in ecosystem. Bioaccumulation of these heavy metals in man, animals and plants result in metal poisoning.
(Audu and Lawal, 2005). Different workers have reported the presence of some heavy metals in some medicinal plants (Zahra *et al*., 2011).

Due to the vast applications in industrial and pharmaceutical processes and the high rate of consumption of Neem products by man and animals, there is a need to continuously investigate the toxicity of different parts of this plant. Most of these neem trees are planted along our major streets and highways in the urban city. Heavy metal contamination has been known to be associated with plants either as a result of uptake from soil or from atmospheric dispersion due to vehicular or human activities. This study is aimed at evaluating some heavy metal content in neem leaves planted along some major highways in Minna Metropolis.

Materials and Methods

Sampling

Neem *(Azadirachta indica)* leaves were collected from three (3) Different locations Along each of these routes, Bosso Road, Angwan Daji Road, Old Airport Road, and Mobil Road of Minna Metropolis, noting the types of activities being carried out within the vicinity of sampling point. The samples were collected and wrapped in a brown envelope each and labeled accordingly and sent to the laboratory for further analysis.

Sample preparation

Freshly collected samples of *Azadirachta indica* leaves were then dried at room temperature and then grounded into powder using a laboratory Mortar and Pestle to reduce dimension, sieved in order to reduce the particle size and then kept in an air tight screw cap plastic containers ready for digestion

Digestion method

Wet digestion method was used and the digestion was carried out in triplicate for each sample by weighing 1.0g of the powdered sample into 100cm^3 Kjeldahl flasks followed by the addition of 4cm^3 of perchloric acid and 25cm^3 of nitric acid and was left to stand for 24 hours under a fume hood.

The mixture was heated at 70^{0}C for 40 minutes and then, the heat was increased to 120^{0}C and the heating continued until solution becomes clear with disappearance of white fumes indicating the completion of the digestion process. The digest was diluted with 10cm^3 of distilled water and boiled for 15 minutes. The resulting solution after cooling were each filtered into a 100cm^3 volumetric flask and diluted to the mark with distilled water. This was then transferred into screw capped polyethylene bottle and stored for further analysis

Atomic Absorption Spectrophotometry

Mn, Cu, Zn, Fe, Pb, Cr and Cd were determined using Atomic Absorption Spectrophotometer (S4 Atomic Absorption Spectrophotometer Thermo electron) with a digital readout system.

Results

The results obtained from the study are shown in tables 1-4. From table 1, the result from the analysis showed that the concentration of Mn along the route ranged from 27.5±3.24 to 37.0±4.50μg/g, and that of Fe ranges from

303.0±12.23μg/g to 765.0±19.27μg/g respectively, with B_C sampling point having the highest concentration for both metals, while the lowest concentration was obtained from B_A sampling point. The concentration of Cu ranged from 6.6±0.14 to 12.0±1.05μg/g with the highest concentration recorded in B_C sample and the lowest in B_B sample. The concentration of Zn ranged from 73.0±6.48 to 113.0±8.37μg/g with the highest concentration obtained from B_B and the lowest from B_C sample, while Cr, Cd, and Pb were not detected in all the samples along this route.

Table 1: Concentrations of Heavy metals in Neem leaves along Bosso Road (*μg/g)*

Samples	Mn	Fe	Cu	Zn	Cr	Cd	Pb
B_A	27.5 ± 3.24	303.0 ±12.23	10.8 ± 0.16	88.3 ± 5.60	ND	ND	ND
B_B	20.5 ± 2.32	335.0 ± 14.30	6.6 ± 0.14	113.0 ± 8.37	ND	ND	ND
B_C	37.0 ± 4.50	765.0 ± 19.27	12.0 ±1.05	73.0 ± 6.48	ND	ND	ND

Key: B_A = Sampling point A along Bosso road; B_B = Sampling point B along Bosso road

B_C = Sampling point C along Bosso road

Table 2 shows the mean concentration of trace metals in samples obtained along Angwan Daji Road. The concentration of Mn ranged from 14.5 ±1.15 to 84.5 ±7.26μg/g and Fe from 355.0 ±13.27 to 634.0 ±18.34μg/g with the highest concentration obtained from A_C sample, while the lowest concentration was obtained from A_A sample. The concentration of Cu ranged from 5.8 ± 0.55 to 15.6 ±1.35μg/g with the highest concentration recorded in U_B sample and the lowest was in A_A sample. The concentration of Zn ranged from 77.0 ± 6.28 to 284 ± 14.15μg/g with the highest concentration obtained from A_B and the lowest from A_A sample. Cr, Cd, and Pb were not detected in all sampling points along this route.

Table 2: Concentrations of Heavy Metals in Neem Leaves along Angwan Daji Road (*μg/g*)

Samples	Mn	Fe	Cu	Zn	Cr	Cd	Pb
A_A	14.5 ±1.15	355.0 ±13.27	5.8 ± 0.55	77.0 ± 6.28	ND	ND	ND
A_B	84.5 ± 7.26	454.0 ± 16.25	15.6 ±1.35	284.0 ± 14.1	ND	ND	ND
A_C	71.0 ± 6.17	634.0 ±18.34	14.0 ±1.22	159.0 ±10.20	ND	ND	ND

Key: A_A = Sampling point A along Angwan Daji road; A_B = Sampling point B along Angwan Daji road

A_C = Sampling point C along Angwan Daji road; ND = Not detected

The result for the mean concentrations of heavy metals in Neem leaves along Old-Airport Road table 3, showed that the concentration of Mn ranged from 36.5 ± 2.65 to 57.5 ± 3.06μg/g, Fe from 424.0 ±16.09 to 532.0 ±17.55μg/g, with the highest concentration obtained from O_C sample while the lowest concentration was obtained from O_A sample. The concentration of Cu ranged from 9.0 to 40.0μg/g with the highest concentration recorded in O_B sample and the lowest in O_C sample. The concentration of Zn ranged from 59.4±3.50 to 361.0±12.53μg/g with the highest concentration obtained from O_A and the lowest from O_B sample. Also Cr, Cd, and Pb were not detected in all the samples along this route.

Table 3: Concentrations of Heavy metals in Neem leaves along Old-Airport Road (*μg/g*)

Samples	Mn	Fe	Cu	Zn	Cr	Cd	Pb
O_A	36.5± 2.65	424.0± 16.09	23.0± 2.07	361.0±12.53	ND	ND	ND
O_B	57.5± 3.06	491.0± 16.40	40.0± 3.35	59.4±3.50	ND	ND	ND
O_C	47.0± 2.85	532.0± 17.55	9.0± 0.65	175.0±8.32	ND	ND	ND

Key: O_A = Sampling point A along Old Airport road; O_B = Sampling point B along Old Airport road

O_C = Sampling point C along Old Airport road; ND = Not detected

The mean concentrations of heavy metals in Neem leaves along Tunga Road are shown in table 4. The result showed that the concentration of Mn ranged from 35.0 ±3.25 to 65.0 ± 5.06μg/g. The concentration of Fe range from 370.0±10.05 to 748.0±18.15μg/g with the highest concentration obtained from T_C sample while the lowest concentration was obtained from T_B sample. The concentration of Cu ranged from 7.0±1.05 to 8.5±1.10μg/g with the highest concentration recorded in T_B sample and the lowest in T_A and T_C sample. The concentration of Zn ranged from 65.0±5.60 to 107.0±8.35μg/g with the highest concentration obtained from T_A and the lowest from T_C sample. Cr, Cd, and Pb were also not detected in all the samples along this route.

Table.4: Concentrations of heavy Metals in Neem Leaves along Tunga Road (*μg/g*)

Samples	Mn	Fe	Cu	Zn	Cr	Cd	Pb
T_A	50.0 ± 4.36	611.0 ± 16.20	7.0 ± 1.05	107.0 ± 8.35	ND	ND	ND
T_B	35.0 ± 3.25	370.0 ± 10.05	8.5 ± 1.10	83.0 ±7.20	ND	ND	ND
T_C	65.0±5.06	748.0 ±18.15	7.0 ± 1.20	65.0 ± 5.60	ND	ND	ND

Key: T_A = Sampling point A along Tunga road; T_B = Sampling point B along Tunga road

T_C = Sampling point C along Tunga road; ND = Not detected

Discussion

From the result obtained from the analysis, the highest concentration of Mn along Bosso Road was (37.0 ± 4.50μg/g) which is lower than those obtained from other sampling routes, while that of Angwan Daji had highest Mn concentration of (84.5 ± 7.26μg/g). This can be attributed to the sewage sludge that may have percolated into the soil and some of the smelting and vehicular activities taken place in this route and sampling point. The major sources of manganese in soil are fertilizers, sewage sludge and ferrous smelters. Plants could uptake this metal through transpiration and get deposited in some vital tissues of the plant. The result compared with that reported by Sumayya *et al*., (2010), of 90.6.μg/g in *Lagenaria scicerrana standl* fruits. While Zahra et al., (2011) reported lower concentration range of 34.57 to 57.30μg/g in *Murraya koenigii*.

The concentrations of Fe in samples obtained along Bosso road (table 1) are 303.0 ±12.23, 355.0 ±13.27, and 765.0 ±19.27μg/g respectively. These results are lower than other sampling points. Tunga road had highest Fe concentration of 611.0 ±16.20, 370.0 ±10.05, and 748.0 ±18.15μg/g compared to other sampling routes. This could be attributed to high Automobile activities, human activities like Mechanic and sales of household materials along this route. The result when compared with that reported by Zahra *et al*., (2011), of 100μg/g Fe in leaves of *Trachyspermum ammi*, *Brassia rapa*, *Foeniculum vulgare*, and *Brassica oleracea*, respectively, is higher. The variation could be attributable to differences in the activities being carried out around the sampling point and variation in species of plant. This may have resulted in their ability to accumulate these metals in their tissues. While Sumayya *et al*., (2010), also reported high Fe concentration of between 885,60μg/g in *Mentha spicata* and 90.58μg/g in *magnifiera indica.* plant.

The concentration of Cu from all the sampling points ranged from 5.8 ± 0.55 to 40.0 ± 3.35µg/g, with the lowest concentration obtained from Unguwan Daji road and highest from Old-Airport Road. The concentration of Cu in the samples can be attributed to human activities such as vulcanizing, sales of petroleum products, disposal of spare parts. From this study, the concentration of Cu of between 5.8 ± 0.55 to 40.0 ±3.35µg/g is within the permissible level of 40µg/g as recommended in plants by WHO (2007). Thus implying that the Cu concentration of the Neem leaves studied is within the tolerable limit that will not be hazardous to health

Generally, the concentration of Zn ranged from 65.0 ± 5.60 to 361.0 ±12.53µg/g. Samples obtained along Tunga Road had lower concentrations of Zn compared to the other routes, while samples from Old-Airport Road have higher concentrations. This can be attributed to high pollution of the area which is caused by human activities from the un-controlled disposal of used machine parts and other metallic scraps around the sampling points. Compared to other workers, higher concentration of Zn of 43.5µg/g in *B. diffusa* to 495.0µg/g in *C. anisata*) has been reported in plants by Atukorala, (1987). Zahra *et al*., (2011), also reported high Zn concentrations of 53.69µg/g and 53.74µg/g in *Foeniculum vulgare* and *Trachyspermum ammi* respectively. Generally, concentrations of the heavy metals in all the routes studied are in the order of Fe> Zn> Mn> Cu, while Cr, Cd, Pb were not detected in all the samples.

Conclusion

The findings from this study shows that there are variations in metal ion concentration in Neem leaves along the various route studied. But the core heavy metals that could be toxic to health were not detectable in all the samples from various locations indicating that the use of the Neem leaves from these locations directly or indirectly for medicinal purposes or other uses will have no negative health effect to the consumer.

It can therefore be concluded from this study that metal ion concentration in Neem leaves along the various route may not contribute any toxicity or harmful effect to human health when taken orally or in the form of tea or as part of diet.

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