Heavy metal contents of Azidirachta indica collected from Akungba-Akoko (Nigeria)

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SUMMARY

The levels of heavy metals in the tissues (roots, leaves and stems) of Azidirachta indica collected at different spots in Akungba-Akoko, Ondo State (Nigeria) were determined. Five heavy metals namely; cadmium (Cd), cobalt (Co) iron (Fe), lead (Pb) and zinc (Zn) were studied. The tissues were ashed, digested and the digestates were analyzed for the metals using flame atomic absorption spectrophotometer. The levels (ppm) of the heavy metals obtained in the roots, leaves and stems of the plant respectively ranged as follows: Cd (0.14 – 0.34, 0.09 – 0.20, 0.09 – 0.33); Co (4.01 – 5.48, 3.22 – 5.14, 3.84 – 5.40); Fe (82.27 – 137.98, 49.20 – 160.10, 58.33 – 116.62); Pb (0.50 – 0.57, 0.33 – 0.97, 0.28 – 0.48) and Zn (52.07 – 149.94, 52.01 – 113.73, 49.58 – 127.05). Fe has the highest levels while Cd has the lowest levels in all the tissues analyzed. The stems of the plant showed the least bioaccumulation of most metals studied and hence most suitable for medicinal purpose. Metals' concentrations decrease in all the tissues with increasing distance from the highway. There is a growing concern about the physiological and behavioural effects of environmental trace metals in human population, for medicinal purpose therefore, tissues of the plant and other medicinal plant should be sourced at distances very far away from the roadside.

Key words- Heavy metals, Azidirachta indica, tolerable limits, anthropogenic sources, toxic effects, biological tissues.

[Afr J Health Sci. 2010; 16:64-69]

Introduction

Heavy metals are natural components of the earth's crust and they can enter the water and food cycles through a variety of chemical and geochemical processes [1]. Living organisms require trace amounts of some heavy metals, including Co, Cu, Fe, Mn, Mo, V and Zn. Excessive levels of these metals, however, can be detrimental to living organisms. Other heavy metals such as Cd, Hg and Pb have no known beneficial effect on organisms and their accumulations over time in the bodies of mammals can cause serious illness [2]. The contamination of roadside soils and plants with heavy metals arises from a number of sources such as vehicles, road wear, slipperiness control, buildings (heating and corrosion) local building activities, and pollution from local industries. The accumulation of the metals in topsoil is greatly influenced by traffic volumes and motor vehicles, which introduce a number of toxic metals into the atmosphere [3].

Heavy metal pollutants are of significant ecological/environmental concern because they are not biodegradable and have long half-lives in the soil, thus predicating far-reaching effects on biological systems including soil microorganisms and soil biota [4, 5]. These metals also are accumulated when plants and crops cultivated along major roads are consumed by man and animals especially livestock, either directly or indirectly [4]. This accumulation may fast reach lethal levels quickly [6]. Analyses of roadside soils

and plants revealed that they contain elevated levels of the heavy metals [7]. Although, there have been a considerable number of studies on the concentrations of heavy metals in roadside soils and plants, the vast majority have been carried out in developed countries with long histories of industrialization and extensive uses of leaded gasoline [8].

Azadirachta indica has many medicinal values, particularly among the Africans, and these have been reported [9, 10, 11, 12, 13]. Plants located along highways have been reported contaminated with heavy metals to various extents depending on their distance from the highway. The closer the plants are to the highways, the higher the degrees of heavy metal contamination and vice versa [14, 15, 16].

Akungba-Akoko in the recent past has witnessed, on daily basis, heavy vehicular movements because of its strategic location serving as a major link way between the south-western and the northern parts of Nigeria. Due to the medicinal values of Azidirachta indica, many rural dwellers depend on it for the treatment of various ailments including malaria fever, a notorious tropical disease. In spite of the many therapeutic uses of this plant however, scanty information is available in the literature on the levels of heavy metals build up in the tissues of the medicinal plant. Consequently, I found it imperative to investigate the concentrations of five heavy metals (Cd, Co, Fe, Pb and Zn) in the tissues of the plant collected from Akungba-Akoko (Nigeria) as a step in risk assessment with a view to determining the extent of exposure to toxicity associated with heavy metals contamination.

Materials and methods

Sample Collection

The leaves, the stems and the roots of the matured plants were carefully and randomly collected for analysis. Samples were collected at distances of 2.0, 5.0, 10.0, 20.0 and 50.0m away from the highway. Upon collection, the samples were washed with tap water, rinsed thoroughly with distilled water, and transported in clearly labeled polythene bags to the laboratory. Only fresh and matured samples of Azidirachta indica in prime conditions were collected in order to produce good quality dried products [17].

Digestion and Analysis of Samples

The tissues of the plant were dried in an oven at 105°C for 24 hrs until they were brittle and crisp [18]. At this stage, no microorganism can grow and care was taken to avoid any source of contamination. The dried samples were grinded into fine particles using acid washed mortar and pestle. The procedure according to [19] was used for the digestion of the plant samples. Triplicate digestion of each sample together with blank digestion without the plant sample was carried out. The quantitation of metallic contents of the digestates was carried out using Alpha 4 serial number 4200 model of the flame atomic absorption spectrophotometer.

Quality Assurance

Quality control test was conducted on the plant samples in order to evaluate the experimental procedures and the efficiency of the flame atomic absorption spectrophotometer. This was done by spiking the pre-digested plant samples with Thermo Jarell Ash standard solution. The spiked samples were then digested as the sample procedure used for plant samples as earlier discussed.

Results and Discussion

Method Validation

The validity of the procedures used for sample treatment and analysis was tested by spiking experiment. The recoveries of the plant samples were 90.0, 92.8, 98.5, 96.8 and 98.2% for Cd, Co, Fe, Pb and Zn respectively. Zn has the highest recovery overall. The recovery results were in good agreement with the expected values. Therefore, the recovery test and the reproducibility of the method were found satisfactory to validate the experimental protocol.

Concentration of heavy metals in plant samples

The concentration (mean ± SD) in ppm of Cd, Co, Fe, Pb and Zn in the roots, stems and leaves of Azidirachta indica are respectively presented in Tables 1, 2 and 3. From the results in Tables 1 - 3, it can be observed that the concentration of Cd ranged between 0.14±0.03 – 0.34±0.03ppm; 0.09±0.03 – 0.33±0.04ppm and 0.09±0.03 – 0.20±0.03ppm respectively in the roots, stems and leaves of the plant. The levels of Cd recorded in the present study compared favourably with those reported by other authors in similar studies [20, 21] but were lower than those reported by [4] as shown in Table 4. Furthermore, the levels of Cd recorded in this study were lower than the critical concentrations proposed for the metal in plants [21]. The tissues of Azidirachta indica therefore showed lower Cd

Table 1: Concentrations of heavy metals (mean ± SD) in pm in the roots of Azidirachta indica.

Sample Codes	Cd	Co	Fe	Pb	Zn
RS1	0.34±0.03	5.48±0.28	137.98±1.39	0.57±0.03	149.44±0.62
RS2	0.24±0.04	5.14±0.14	102.23±0.33	0.54±0.06	113.95±1.48
RS3	0.23±0.03	4.95±0.11	98.87±0.52	0.53±0.04	71.98±0.68
RS4	0.15±0.04	4.04±0.28	95.76±0.93	0.51±0.06	54.58±1.41
RS5	0.14±0.03	4.01±0.11	82.27±0.38	0.50±0.11	52.07±0.01

Note: R = samples taken from the roots of the plant;
S1 = 2.0m, S2 = 5.0m, S3 = 10.0m, S4 = 20.0m and S5 = 50.0m away from the highway.

Table 2: Concentrations of heavy metals (mean ± SD) in ppm in the stems of Azidirachta indica.

Sample Codes	Cd	Co	Fe	Pb	Zn
BS1	0.33±0.04	5.40±0.28	116.62±0.88	0.48±0.10	127.05±0.67
BS2	0.28±0.03	4.91±0.18	89.35±0.49	0.44±0.03	123.32±0.59
BS3	0.23±0.10	4.54±0.06	84.14±0.20	0.38±0.11	97.81±1.15
BS4	0.18±0.03	4.52±0.14	72.22±0.31	0.37±0.07	69.71±1.00
BS5	0.09±0.03	3.84±0.23	58.33±0.47	0.28±0.13	49.58±0.47

Note: B = samples taken from the stems of the plant;
S1 = 2.0m, S2 = 5.0m, S3 = 10.0m, S4 = 20.0m and S5 = 50.0m away from the highway.

contamination in comparison with results reported in similar studies.

The concentration of Co obtained in the roots, stems and leaves of the plant respectively ranged between 4.01±0.11 – 5.48±0.28ppm; 3.84±0.23 – 5.40±0.28ppm and 3.22±0.07 – 5.14±0.08ppm.The Co levels recorded in the present study were higher than those reported by [20] and compared favourably with those of [21] but were lower than the critical levels proposed by [21] as shown in Table 4. The concentration recorded for Fe ranged between 82.27±0.38 – 137.98±1.39ppm in the roots; 58.33±0.47 – 116.0.88ppm in the stems

Table 3: Concentrations of heavy metals (mean ± SD) in ppm in the leaves of Azidirachta indica.

Sample Codes	Cd	Co	Fe	Pb	Zn
LS1	0.20±0.03	5.14±0.08	160.10±1.24	0.97±0.14	113.73±1.03
LS2	0.19±0.06	5.30±0.18	88.89±0.84	0.63±0.04	104.95±0.87
LS3	0.19±0.03	3.65±0.01	84.12±0.80	0.38±0.13	99.28±0.71
LS4	0.14±0.06	3.62±0.11	53.30±1.00	0.34±0.07	98.59±0.87
LS5	0.09±0.03	3.22±0.07	49.20±0.47	0.33±0.13	52.01±0.59

Note: L = samples taken from the leaves of the plant;
S1 = 2.0m, S2 = 5.0m, S3 = 10.0m, S4 = 20.0m and S5 = 50.0m away from the highway.

Table 4: The levels of heavy metals (ppm) in tissues of Azidirachta indica compared with other studies worldwide.

Metals	Akungba-Akoko	London(WAL)	Glasgow	Hong Kong	Kaduna (Nigeria)
Cd	0.09 - 0.34	0.60	0.10 - 2.40	N.D.	4.88 - 14.93
Co	3.22 - 5.48	0.50	0.02 - 10.00	-	-
Fe	49.20 - 160.10	140.00	0.02 - 50.00	-	-
Pb	0.28 - 0.97	2.70	0.20 - 30.00	134.00	0.00 - 32.37
Zn	49.58 - 149.44	100.00	1.00 - 400.00	124.00	27.78 - 185.19
Reference	Present Study	[20]	[21]	[7]	[8]

Note: ND = not detectable; WAL = world average level.

and 49.20±0.47 – 160.10±1.24ppm in the leaves of the plant. Table 4 showed that the levels of Fe recorded in the present study compared favourably with those earlier reported by other authors in similar studies [20, 21]. The highest value recorded for the metal was lower than the highest critical concentration proposed for the metal [21].

Pb recorded concentration that ranged between 0.50±0.11 – 0.57±0.03ppm in the roots; 0.28±0.13 – 0.48±0.10ppm in the stems and 0.33±0.13 – 0.97±0.14ppm in the leaves of the plant. The Pb contents recorded in the tissues of Azidirachta indica in the present study were lower than those reported in similar studies carried out elsewhere [7, 8, 20, 21]. The tissues therefore showed lower Pb contamination in comparison with other results reported in similar studies. The concentration of Zn recorded respectively in the roots, stems and leaves of the plant ranged as; 52.07±0.01 – 149.44±0.62ppm; 49.58±0.47 – 127.05±0.67ppm and 52.01±0.59 – 113.73±1.03ppm. The concentration of Zn recorded in the present study compared favourably with those reported by other authors in similar studies. The highest Zn level recorded was far lower than the highest critical level proposed by [21] for the metal. The least concentrations of all the metals were recorded in the tissues collected at 50.0m away from the highway while the highest concentrations of the metals occurred in the tissues collected at 2.0m away from the highway. Of all the metals investigated, Fe has the highest concentration while Cd has the lowest concentration in all the tissues studied. The bioaccumulation of Cd, Co and Zn in the roots (R), stems (B) and leaves (L) of Azidirachta indica was in the order R > S > L while the bioaccumulation of Fe and Pb was in the order L > R > S. The roots of the plant showed highest bioaccumulation of most metals studied followed by the leaves while the stems showed the least bioaccumulation, hence, for medicinal purpose, the stems of the plant should be preferably used.

Correlational calculations between pairs of heavy metals in the tissues of Azidirachta indica as presented in Table 5 gave positive (direct) correlation coefficients that were very significant ($p<0.05$) for the metals. The high correlation coefficients obtained for the pairs of heavy metals indicate that significant heavy metal pollution from extraneous source(s) has taken place.

Table 6 showed correlation coefficients carried out between levels of heavy metals in the tissues of Azidirachta indica. As can be observed, positive correlations, that were significant ($p<0.05$) existed in all cases. The high coefficients recorded reflect similar sources of the metals in all the tissues studied.

Table 5: Correlation coefficients between pairs of heavy metals in the tissues of Azidirachta indica.

Metal pairs	Roots	Stems	Leaves
Cd and Co	0.9571	0.9777	0.7732
Cd and Fe	0.9387	0.9587	0.7494
Cd and Pb	0.9926	0.9893	0.6619
Cd and Zn	0.9484	0.9779	0.9194
Co and Fe	0.8132	0.9662	0.9194
Co and Pb	0.9529	0.9922	0.9722
Co and Zn	0.9095	0.9215	0.7625
Fe and Pb	0.9494	0.9455	0.9555
Fe and Zn	0.9089	0.9099	0.6719
Pb and Zn	0.9663	0.9536	0.6234

Table 6: Correlation coefficients between concentrations of heavy metals in the tissues of Azidirachta indica.

Metals	rRB	rRL	rBL
Cd	0.9243	0.8422	0.9496
Co	0.8628	0.8726	0.9555
Fe	0.9718	0.9733	0.9721
Pb	0.9350	0.9432	0.8587
Zn	0.9056	0.6812	0.8471

Note: rRB = correlation coefficient between concentration of heavy metals in roots and stems;
rRL = correlation coefficient between concentration of heavy metals in roots and leaves;
rBL = correlation coefficient between concentration of heavy metals in stems and leaves.

In other to determine the relationship between the distance from the roadside and the levels of heavy metals in the tissues of the plant, correlation calculations were performed and the results were presented in Table 7. It can be observed from the results that negative

Table 7: Correlation coefficients between concentration of heavy metals in the tissues of Azidirachta indica and the distance from the highway.

Metals	Roots	Stems	Leaves
Cd	-0.7872	-0.9510	-0.9772
Co	-0.8397	-0.9119	-0.7860
Fe	-0.7386	-0.8492	-0.7066
Pb	-0.8302	-0.9395	-0.6505
Zn	-0.7334	-0.9208	-0.9789

correlations that were significant ($p<0.05$) existed between distance from the roadside and the levels of all the metals in all the tissues of the plant. The negative correlations recorded indicate that inverse relationship existed between the levels of the metals in the tissues of Azidirachta indica and the distance from the edge of the road. Hence, exposure to vehicular activities is one of the major sources of heavy metal contamination in the studied area. The inverse relationship recorded in the present study was in agreement with those reported by other authors in similar studies [7, 14, 15, 16].

The roots, stems and leaves of Azidirachta indica have been used severally by Africans as herbal remedies for curing many ailments [10, 11]; therefore, for medicinal purposes, the tissues of the plant should be sourced at very far away distance from the highway where there is remote exposure to vehicular activities and where metals' levels in the plant tissues are infinitesimally low and within tolerable limits recommended for human consumption.

Concentrations obtained for Cd, Co, Pb and Zn in all the tissues of the plant were found to be within the tolerable limits recommended for plants, while Fe concentrations exceeded maximum tolerable limits recommended for plants [20, 21]. However, none of the concentrations recorded for all the metals in the tissues of Azidirachta indica exceeded the critical levels proposed for plants. Co, Fe and Zn are essential micronutrients and their seemingly high concentrations in the analyzed tissues were therefore not alarming. Cd and Pb are heavy toxic metals, but their concentrations in all the tissues studied were below the levels considered toxic [21]. There is a growing concern about the physiological, biochemical and behavioural effects of environmental trace metals in human populations. The toxicities of Cd, Pb and other heavy metals at high levels of exposures are well known, but the concern of today is the possibility that continual exposure by the use of the analyzed tissues as herbal remedies may result to gradual accumulation of these metals in the human systems and may lead to adverse health effects.

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Conclusion

The concentrations recorded for Cd, Co Pb and Zn in the roots, stems and leaves of Azidirachta indica fell within the recommended tolerable limits. Fe concentration exceeded the maximum tolerable limits recommended for plants. None of the concentrations recorded for the metals in the plant tissues exceeded the critical levels recommended for plants. Stems of the plant showed the least bioaccumulation of most metals studied and hence most suitable for medicinal purpose. Reciprocal relationship existed between the levels of heavy metals in the plant tissues and the distance of the plant away from the highway, hence, for medicinal purposes, plant tissues should be sourced at very far away distance from the highway where traffic effect is very low.

Acknowledgement

The contributions of Mr. Adisa I. A. of the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria, and Miss Kemi Olanisebe of the School of Nursing, Ado-Ekiti, Nigeria, toward the success of this work, were greatly appreciated.

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