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Evaluation of Heavy Metal Contamination of Soils alongside Awka- Enugu Road, Southeastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author DEE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CBI carried out the field and laboratory studies. Author JNO managed the analyses of the study. Author AIO managed the literature searches, carried out the spatial interpretation of this work and helped with the literature review. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Fourteen top soil samples were collected from old Awka-Enugu road passing through Obioma, Nsude, Ngwo and Abor communities. Samples were taken from a depth of 10-15cm after making a hole with a pick axe. Total concentrations of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), cobalt (Co), and chromium (Cr) were determined using a Buck 210 VGP atomic absorption spectrophotometer (AAS).Soil contamination was evaluated with pollution index (Pi) and index of enrichment factor (EF) concept. The enrichment factor of copper (Cu) was observed to be generally low across the study area. This implies that Cu toxicity in the area is not significant. However, the enrichment and pollution indices of cadmium (Cd) and lead (Pb) were observed to be high within the study area.The study area was observed to be highly contaminated by cadmium and lead but moderately contaminated with respect to nickel, zinc and chromium. The enrichment and pollution trends of the metals in descending order shows Cd > Pb > Ni > Zn > Cu > Cr > Co. In summary, the total heavy metal concentrations of soils studied were slightly higher than previously reported levels for similar soils which suggest some degree of heavy metal pollution.

Keywords: Pollution index; enrichment factor; heavy metals; contamination; health risk level.

1. INTRODUCTION

Surfaces such as pavements and road surfaces in urban environments usually contain an accumulation of fine solid particles on them. These particles deposited on these surfaces are commonly referred to as street dust, road deposited sediments (RDS) or road dust. Road deposited sediments are usually a complex mixture of fine solid particles and pollutants obtained from a wide range of urban and industrial sources and processes. Their sources are either as a result of human activities (vehicle tyre and body wear, vehicular exhaust emissions brake-lining materials, road salt, building and construction materials or soil material particles, litter obtained from plant and leaf and atmospheric deposition [1-5]. Roads are known as the second largest non-point source of creating pollution in urban environments. Road and highway surfaces are impervious, and serve as temporary sinks for various types of pollutants that are washed off during rainfall to the surrounding environments [6-8]. Road surfaces receive varying amount of heavy metals by the process of atmospheric deposition, sedimentation, impaction and interception [9]. In urban environments, the top soils and road deposited sediments (RDS) or road dust are indicators of heavy metal contamination from atmospheric deposition. Industries, traffic, mining activities, smelters and construction are some of the main anthropogenic sources of this heavy metal pollution. The traffic source includes vehicles (tyre wear, brake linings, fuel combustion, etc.) while road infrastructure (pavement wear, corrosion of galvanized steel crash barriers, etc) contribute the rest. It has been reported that pollutants such as arsenic, cadmium, chromium, copper, nickel, lead and zinc generally have high concentrations on road surfaces, roadside soils and particulate matter due to heavy traffic, and most often affect the environmental air quality [10].

Roads in the study area play a major role in stimulating socio-economic progress and development. Major activities of the inhabitants of the study area include sales of vehicle parts, repairs and scavenging of useful motor parts from scrapes. Wastes emanating from such activities could lead to contamination of surficial materials including soils. Contamination by heavy metals in any environment is a major concern because of their toxicity, threat to life and the

ecosystem. Automobile mechanic workshop wastes have been reported by earlier scholars for the elevated concentrations of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) in soil profiles in the vicinity of automobile waste dumps in Nigeria [11]. Remarkable metal contamination especially lead pollution has also been attributed to automobile emissions in the roadside ecosystems [12]. Most researchers have reported the influence of the traffic load on heavy metal contents in top soil and their variability with distance [13-15].

Viard et al. [7] reported that the deposition and the levels of metals in surficial soils decrease with increasing distance from the highways. They study also revealed that the impact of the highway was clear up to 320 m at least for lead, but the most important contamination was observed near the road. Nabuloa et al. [16] reported that leaves of roadside crops can bioaccumulate trace metals at high concentrations causing serious health risk to consumers of such products. Hence, accurate evaluations of the heavy metal concentrations of polluted soils are required to assess the potential ecological risk of affected areas. The potential health risks and high levels of heavy metal pollution measured in a number of cities of the world have therefore generated a lot of interest in heavy metal pollutions in road deposited sediments. Roadways and automobiles are presently considered as the largest sources of heavy metals in the urban environment. The objectives of this study are to assess the heavy metal contamination in roadside top soils along the old road passing though Obioma- Nsude – Ngwo and Abor areas of Enugu State, Southeastern Nigeria and to compare the observed levels of pollution in the area with established standards of unpolluted soils. It is envisaged that the study would provide background data for subsequent investigation of the environment, biological dose response and effect on human health.

1.1 Background Geology of the Study Area

The study area lies within longitudes $7°20'$ – $7^{\circ}22'$ E and latitudes $6^{\circ}21'$ – $6^{\circ}30'$ N (Fig. 1). The Night Mile Corner, Ngwo-Uno area, has been the centre of industrial and commercial activities in recent times, particularly those industries that require viable and efficient water supply systems. This is because the study area has a high

Fig. 1. Location and geological map of the study area

groundwater potentials when compared to other surrounding areas which is often attributed to the local hydrogeological characteristics of the area. The study area is underlain by the Ajali Formation which is known for its huge aquiferous potentials. The Ajali Formation is made of well sorted, massive, coarse –grained, white sandstones with occasional intercalations of thin beds of white shales and clays. Rivers Ekulu, Iva, Oroho and Nyaba take off from the base of the Ajali Formation on the Enugu escarpment descending through the underlying Mamu Formation into Enugu town (Fig. 1). The Mamu Formation trends northeast (N-E) of the study area (Fig. 1) and comprises of alternations of sandstones, dark-blue or grey shales, sandy shales and mudstones with local coal seams at various horizons [17].

2. MATERIALS AND METHODS

Fourteen (14) top soil samples were systematically collected along Awka-Enugu old road passing through Obioma- Nsude - Ngwo - Abor communities which stretch about 17 kilometers (Fig. 1). The sampled soil layer partly contains humus with completely decomposed non-identifiable organic materials. Sample

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numbers 4, 5, 6, 7, 9, 10, 11 and 13 were collected from the edge of the road while sample numbers 1, 2, 3, 8, 12, and 14 were collected farther away off the road (3-150 m). Each sample was taken from a depth of 10-15cm after making a hole with a pick axe from the surface. Site locations were recorded and geo-referenced using a standard Garmin e Trex global positioning system (GPS) instrument. The sample coordinates, names and descriptions are presented in Table 1. Soils collected varied in texture, colour and composition from clay to sand with some samples having gray, red and brown colours. A good vegetation cover was noticed with *Imperata cylindrica* (tall grasses) being dominant. Soil pH which is a measure of the acidity or basicity considered as a master variable was measured by the use of an electronic pH-meter (Table 2).

The soil samples were air-dried, disaggregated and mechanically sieved using 100-mesh screen. Samples are considered acceptable if 100% passes through 100-mesh screen [18] and also to ensure homogeneity prior to digestion [19]. Digestion of soil samples were carried out according to standard procedures as described by [20] and [21], where 0.2 grams of each

sample was weighed and dried using crucibles. The samples were digested in acid mixtures of 5 ml nitric acid, 3 ml perchloric acid and 2 ml hydrofluoric acid [20]. They were placed on hot plates for ten minutes and later transferred to a fume chamber where they were allowed to cool. The cooled solutions were made up to 250 ml in volumetric flasks with de-ionized water. The obtained solutions were later used for the measurement of heavy metals including Lead (Pb), Copper (Cu), Chromium (Cr), Nickel (Ni), Cadmium (Cd), Zinc (Zn) and Cobalt (Co) using the Buck 210 VGP atomic absorption spectrophotometer.

3. RESULTS INTERPRETATION AND DISCUSSION

Heavy metal concentrations are expressed in milligram per kilogram (mg/kg) of dry weight of the soil samples. The summary of parameters of the analytical data of the soil samples are shown in Table 2. The enrichment factor (EF) and pollution index (PI) are presented in Tables 3 and 4 respectively while Table 5 shows the range and mean values of heavy metal contents (mg/kg) of soil samples and related established background values from [22-25] and [26] for non-polluted soils.

The concentrations (mg/kg) of trace metals determined from the soil samples of the area are shown in Table 2 and Fig. 2 while Table 5 shows the range of values used for comparison. Cadmium (Cd) in unpolluted soils is normally in the concentration range of $0.01 - 0.35$ mg/kg according to tolerable standards by [22]. However, cadmium in Table 2 ranged from <0.37 to 6.12 mg/kg with mean values exceeding the tolerable limits of 2.00 mg/kg as given by [22], 0.05 mg/kg by [24], 0.15 mg/kg by [25] and 0.08 mg/kg as given by [26]. It implies that Cd has impacted negatively on the agricultural soil and the environment at large. High cadmium concentrations in the study area may be a direct result of anthropogenic sources. Similarly, chromium ranged from < 1.12 to 5.12 mg/kg with an average value of 1.99 mg/kg. At sampling sites 3, 6, 7 and 11, chromium was not detectable. Chromium values and the mean value are much lower than the referenced standard levels [22-26]. Copper varied from 0.12 to 3.75 mg/kg, with a mean value of 1.44 mg/kg. Similarly, copper was lower than the established tolerable limits. This means that Cr and Cu may not have had any significant effect on the environment. Nickel ranged from 0.87 – 11.87 mg/kg with an average concentration of6.11 mg/kg while cobalt ranged from 0.25 to

Table 1. Location of soil samples

Sample	Latitude	Longitude	Remarks
location	N(degrees)	E (degrees)	
Obioma -1	06°29'748"	07°25' 186"	Fine grained, friable loose sand.
Obioma -2	06°21'803"	$07^{\circ}25'$ 034"	Well sorted sand weathered to reddish-brown coloured clayey soil.
Obioma -3	06°22'299"	$07^{\circ}25'$ 016"	Heavily leached dark loam soil.
Nsude-4	06°22'748"	07°24' 189"	Fine grained friable sand and ironstone weathered to reddish laterite with concretions.
Nsude-5	06°23'334"	$07^{\circ}24'$ 109"	Weathered brown/reddish ironstones, quartz pebbles which favours extensive growth of vegetation.
Nsude-6	06°24'497"	07°24' 133"	Light brown, fine grained and well sorted sand.
Ngwo-7	06°25'406"	07°26' 181"	Loam soil with thick vegetation cover with tall grasses.
Ngwo-8	06°25'019"	07°26' 267"	Ironstone rubbles weathered to gray and brown coloured laterite.
Ngwo-9	06°26'536"	07°26' 439"	Loose friable, gray coloured well sorted sand.
Ngwo-10	06°27'023"	07°26' 422"	Brown weathered ironstones, quartz pebbles.
Abor-11	06°27'496"	07°25' 350"	Heavily leached/weathered to reddish-brown coloured laterite and loam with scanty vegetation cover.
Abor-12	06°28'474"	07°25' 414"	Silty and dark coloured sand
Abor-13	06°21'803"	07°25' 034"	Ironstone boulders weathered to red coloured soil, favours growth of tall grasses.
Abor-14	06°29'748"	07°25' 186"	Unconsolidated weathered sand on a flat terrain dotted with shrubs

1.22 mg/kg with 0.43 mg/kg as the average value. Cobalt was not detected in some locations (Table 2).Average concentrations of Cr (1.99 mg/kg) and Co (0.43 mg/kg) were very low when compared with established limits of [22], [23] and [26] in Table 5. They therefore pose no risk to health and the environment. However, the concentrations of lead (Pb) are slightly below toxic level. Lead concentrations across the study area ranged from 0.50 to 4.75 mg/kg with the highest concentrations revealed at sites 7 and 14. At these two locations, auto mobile mechanic workshops are located close by and are believed to have directly contributed to the high concentrations at the locations. Zinc concentrations ranged from <1.87 to 7.25 mg/kg with an average value of 4.41 mg/kg. Concentration sequence of the examined metals in descending order is presented below: Ni > Zn $> Pb > Cd > Cr > Cu > Co (Table 2).$ The average concentration value of nickel (Ni) of 6.11 mg/kg is considered high but below the 50 mg/kg tolerable limit set by [22]. The toxicity of excess Ni present in the soil could account for the poor growth of weeds at some of the locations. Nickel is carcinogenic and affects the reproductive health of organisms [27-28]. The high level in the study area may be due to aerial deposition, manure and phosphate fertilizer application.

Table 2. Concentrations (mg/kg) of metals in soil samples

Sample	Concentration of metals in mg/kg									
location	C _d	Cr	Cu	Ni	Pb	Co	Zn	рH		
Obioma-1	6.12	1.37	2.87	6.36	4.12	0.62	7.00	6.0		
Obioma-2	5.25	1.50	2.50	1.50	0.87	0.00	7.00	6.0		
Obioma-3	4.75	0.00	0.12	10.37	3.50	0.00	2.87	5.0		
Nsude-4	4.62	4.00	0.12	6.75	1.75	0.00	3.62	6.0		
Nsude-5	2.50	3.00	1.00	2.62	2.25	1.12	4.37	6.0		
Nsude-6	1.12	$0 - 00$	0.75	10.1	1.87	0.62	1,87	6.0		
Ngwo-7	1.12	0.00	0.12	4.00	4.75	0.37	4.00	6.0		
Ngwo-8	0.72	1.12	1.87	0.87	3.25	0.00	6.00	5.0		
Ngwo-9	0.25	3.75	2.25	11.12	0.50	0.37	5.00	6.0		
Ngwo-10	0.00	3.62	0.87	11.00	2.25	0.87	0.00	5.0		
Abor-11	1.50	0.00	3.75	6.62	1.37	0.25	3.50	6.0		
Abor-12	1.62	2.12	0.62	11.8	2.62	0.37	2.62	6.0		
Abor-13	1.12	5.12	1.12	1.62	3.50	0.87	7.25	6.0		
Abor-14	0.37	2.37	2.00	0.75	3.75	0.25	6.25	6.0		

Table 3. Enrichment factors (EF) of soil samples

Sample	Pollution index (Pi) of metals							
location	C _d	Cr	Cu	Ni	Pb	Co	Zn	
Obioma-1	76.50	0.01	0.10	0.10	0.37	0.03	0.09	
Obioma-2	65.62	0.01	0.09	0.02	0.07	0.01	0.03	
Obioma-3	59.37	0.00	0.01	0.17	0.31	0.00	0.03	
Nsude-4	57.75	0.02	0.00	0.11	0.15	0.00	0.05	
Nsude-5	31.25	0.02	0.03	0.04	0.20	0.05	0.06	
Nsude-6	14.00	0.00	0.02	0.17	0.17	0.03	0.02	
Ngwo-7	14.00	0.00	0.00	0.06	0.43	0.01	0.05	
Ngwo-8	9.00	0.00	0.06	0.01	0.29	0.00	0.08	
Ngwo-9	3.12	0.02	0.08	0.18	0.04	0.01	0.06	
Ngwo-10	0.00	0.02	0.03	0.18	0.20	0.04	0.00	
Abor-11	11.75	0.00	0.13	0.11	0.12	0.01	0.04	
Abor-12	20.00	0.01	0.02	0.20	0.23	0.01	0.03	
Abor-13	14.00	0.03	0.04	0.02	0.31	0.04	0.10	
Abor-14	4.62	0.01	0.07	0.01	0.34	0.01	0.08	

Table 4. Pollution index (Pi) of metals in soil samples

Table 5. Average and range values of heavy metal content (mg/kg) of all sampled sites and related to background values from different sources

The concentration of an element in the soil solution is believed to depend on the equilibrium between soil solution and the solid phase, with pH playing a decisive role [29-30]. While discussing the relatively high mobility of heavy metals with regard to pH, [30] considered that in acid soils (pH $4.2 - 6.6$) the elements: Cd, Ni, and Zn are highly mobile, Cr is moderately mobile, and Cu and Pb practically immobile. Soil pH in this study ranged from 5.0 - 6.0 with an average value of 5.7 (Table 2). This is quite acidic, indicating a high potential of acidic leachate generation and confirming the lack of inherent alkalinity producing minerals. Unpalatable situations would in turn affect the larger predators and compel them to move to other places once they lose their food supply. Crops cannot naturally grow well and flourish in a polluted soil. The acidic level may have adverse effect on agricultural soil. Table 3 and Fig. 3 shows the Enrichment Factors (EF) in the sampled soils. Enrichment factor can fully reflect the impact which was brought to the environment by human activities [31]. It is also a convenient measure of geo-chemical trend for making comparisons between an area and overtime [32]. Based on (EF), the health risk level (HRL) is inferred. When the (EF) is less than 1, it indicates that there is no contamination, HRL is zero. When it is equal to one or two, it indicates that the pollution is minimal and HRL is 1. When (EF) lies in between 2 and 5, it indicates a low level and HRL is 2. When (EF) ranges from 5 - 20, HRL is 3. This shows an intermediate level of pollution. When (EF) is 20 – 40, HRL is 4 and it indicates a high level of pollution but when (EF) is greater than 40, HRL is 5, it means that pollution is intense. Average values (Table 3) indicate that the enrichment trend of the metals is in this order: $Cd > Pb > Ni > Zn > Cu > Cr > Co.$ Cobalt and Chromium, each has HRL of 2 and this indicates a low level pollution condition. Nickel, Zinc and Copper are at intermediate

levels of pollution with a HRL of 3. Cadmium and Lead show respective HRL of 5 and 3. Their pollution levels are considered high.

3.1 Pollution Index and Enrichment Factor

Pollution is any process whether natural or manmade which leads to harmful or objectionable increase in the amount of any factor in the environment [27]. Pollution Index (PI) and Enrichment Factor (EF) were employed to assess the pollution of individual metals in the top soils. The enrichment factor estimates presented in Table 3 was computed using the method prescribed by [31] as shown in equation 1 below:

$$
EF = \frac{C_n(sample)/C_{ref}(sample)}{B_n(Background)/B_{ref}(Background)}
$$
 (1)

Where C_n (Sample) is the concentration of the given element in the examined environment, and C_{ref} (Sample) is the concentration of the element in the reference sample. Bn (Background) is the

Fig. 2. Spatial distribution of the concentrations of some heavy metals (mg/kg): (a) Cadmium (Cd) (b) Chromium (Cr) (c) Nickel(Ni) (d) Lead(Pb)

Fig. 3. Spatial distribution of the enrichment factor of some heavy metals: metals: . Spatial distribution of the enrichment factor of some heavy m
(a) Cadmium (Cd) (b) Copper (Cu) (c) Nickel(Ni) (d) Cobalt (Co)

concentration of the element in the background samples in the environment. B_{ref} (Background) is the concentration of the element in the reference background sample environment. Enrichment factor (EF) represents the level of the element in considered space [31]. Pollution Index (Pi) was also used in this study to assess the contamination of individual metals. Similarly, the pollution index (PI) presented in Table 4 and presented in Fig. 4 was calculated using [31] as shown in equation 2 below: background sample environment. Enrichment
factor (EF) represents the level of the element in
considered space [31]. Pollution Index (Pi) was
also used in this study to assess the
contamination of individual metals. Similar

$$
PI = C_i / S_i \tag{2}
$$

Where P_i = Pollution Index, C_i = Concentration of heavy metal in the medium and S_i = relevant standard for this metal [26]. The tolerable limits
set by various established standards of nonset by various established standards of non polluted soils from [22-26] as shown in Table 5 were used as the assessment criteria.

The pollution index (Pi) was introduced as part of assessing pollution levels by considering the joint effects of all polluting metals in soils [33]. Pollution index was extensively used for soil

concentration of the element in the background pollution studies in China [34-36] and proved to a
samples in the environment. E_{nef} (Background) is be simple and effective because it related to a
the concentration of the be simple and effective because it related to a certain quality criterion. The Pollution Index data (Table 4) highlights the pollution status of the soils in the area. Pollution index (Pi) was also adopted here to assess the contamination of individual metals. The pollution index was calculated based on concentration of seven metals namely: Cd, Cr, Cu, Ni, Pb, Co and Zn which are considered significant in the pollution assessment of the area. The pollution index (Pi) was calculated by averaging the ratios of the concentration of the heavy metals to the hazard criteria (tolerable level) using the formula as shown in equation 2 above [31,36]. Based on Pollution Index (Pi), soil contamination levels can be classified into unpolluted $(Pi < 1)$; slightly polluted $(1 \leq Pi < 2)$; moderately $(2 \leq Pi < 3)$ and heavily polluted (Pi \geq 3). The pollution index of Cadmium in unpolluted soils from literature should be less than one. Table 4 shows Cadmium pollution index ranged from 0.00 76.50 with an average value of 27.73 mg/kg. This implies a high pollution level according to the 76.50 with an average value of 27.73 mg/kg. This
implies a high pollution level according to the
criteria of [36-37]. Lead varied from 0.07 mg/kg studies in China [34-36] and proved to
le and effective because it related to a
quality criterion. The Pollution Index data
(a) highlights the pollution status of the
the area. Pollution index (Pi) was also The pollution index was

n concentration of seven

Cr, Cu, Ni, Pb, Co and Zn

d significant in the pollution

rea. The pollution index (Pi)

weraging the ratios of the

heavy metals to the hazard

vel) using the formula as ; moderately $(2 ≤ Pi < 3)$ and $≥$ 3). The pollution index of luted soils from literature an one. Table 4 shows index ranged from 0.00 -

Fig. 4. Spatial distribution of the pollution index of some heavy metals: (a) Chromium (Cd) (b) (Cd) (b) index of Copper (Cu) (c) Lead (Pb) (d) Zinc (Zn)

to 0.37 mg/kg without local enrichment at any of the localities. The average Lead value 0.14 mg/kg is below the toxic limit. All the remaining five metals analyzed were either below detection levels or are very low in pollution index criteria. They ranged from $(0.00 - 0.43)$ mg/kg with averages Cr (0.01) mg/kg, Cu (0.03) mg/kg, Ni (0.09) mg/kg, Co (0.01) mg/kg and Zn (0.05) mg/kg. The pollution trend is in the order: Cd > $Pb > Ni > Zn > Cu > Cr > Co.$ None of the average values exceeded one (Table 4). This, according to the classification of [26], implies no pollution by these metals. Their health risk level is zero and thus does not pose any health risk to humans though may have some implications to soil and associated plants. mg/kg is below the toxic limit. All the remaining
five metals analyzed were either below detection
levels or are very low in pollution index criteria.
They ranged from $(0.00 - 0.43)$ mg/kg with
averages Cr (0.01) mg/kg,

4. SUMMARY AND CONCLUSION

Heavy metal analysis of fourteen top soil samples collected from old Awka-Enugu road were carried out using atomic absorption spectrophotometer (AAS). Result of the analysis revealed that cadmium concentration in the study area ranged from <0.37 to 6.12 mg/kg. High out using atomic absorption
neter (AAS). Result of the analysis
cadmium concentration in the study
from <0.37 to 6.12 mg/kg. High cadmium concentration in the study area may be associated with natural and anthropogenic sources. Similarly, chromium concentrations ranged from \leq 1.12 to 5.12 mg/kg with an average value of 1.99 mg/kg while copper concentrations varied from 0.12 to 3.75 mg/kg, with mean value of 1.44 mg/kg. Nickel concentrations across the study area ranged from 0.87 – 11.87 mg/kg while cobalt ranged from 0.25 to 1.22 mg/kg. Lead concentrations across the study area ranged from 0.50 to 4.75 mg/kg with the highest concentrations revealed at sites 7 and 14 where automobile mechanic workshops are located. Finally, zinc concentrations ranged from <1.87 to 7.25 mg/kg with an average value of 4.41 mg/kg. cadmium concentration in the study area may be associated with natural and anthropogenic sources. Similarly, chromium concentrations an concentrations average value of 1.99 mg/kg while copper concentrations varied from 0.

In conclusion, cadmium concentration was generally high in the examined area compared to the typical range found in unpolluted agricultural soils. The study area has been highly contaminated by cadmium and lead but moderately polluted with respect to nickel, chromium and zinc. Though cadmium and lead chromium and zinc. Though cadmium and lead
concentrations are high, yet their concentrations

are slightly below toxic levels and therefore may not pose serious risk to human health but may have some implications to the soil and associated plants. Copper and cobalt are deficient and pose no risk to the environment. Enrichment Factor (EF) for the elements in the different sites shows significant variability. It is therefore suggested that the (EF) variability is due to anthropogenic activities in these areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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