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Research Article

Probability of Health Risk, Bioaccumulation, and Geochemical Fractions of Toxic Elements in Soils and Vegetables Impacted by Manures in Nigeria

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Abstract: The intensive application of manures for the improvement of plant yield is a global practice by farmers notwithstanding the associated negative effects. The probability of health risk, bioaccumulation, and speciation of arsenic (As), cadmium (Cd), nickel (Ni), and lead (Pb) in soils and vegetables (*Talinum triangulare* and *Telfairia occidentalis*) impacted by untreated wastes from animal farms and inorganic fertilizers used as manures in the oil producing section of Nigeria were investigated. The total metal was determined using spectrophotometric methods while its geochemical fractions were identified following the modified Community Bureau of Reference (BCR) procedures of sequential extraction. The results obtained indicated the following mean concentrations (mg kg^{-1}): 1.64 ± 0.70, 3.17 ± 1.47, 62.22 ± 8.83, and 187 ± 29.73 for As, Cd, Ni, and Pb, respectively. The results revealed that the mean concentrations of all the metals in the studied locations, except As, were above their recommended limits by the Food and Agricultural Organization/World Health Organization (FAO/WHO). The mean values of As and Pb in *T. triangulare* and *T. occidentalis* were also above the acceptable limits. Relatively higher levels of the metals were recorded at the studied locations as compared to the control site. The transfer factors of metals determined were generally lower than one; hence, the human exposure rate to these metals may not be high. The sequential extractions of metal indicated that As and Pb existed in the reducible fraction, Cd in the acid extractable fraction, and Ni in the residual fraction. The contamination factor of the metals revealed that As and Ni belong to the low contamination class, while Cd and Pb fall within the very high contamination category. The study showed that all the studied locations were heavily contaminated, with Uyo being the most polluted site. The principal component analysis identified anthropogenic factor as the main contributor to the presence of these metals in the investigated area. Daily intake rates for these metals via vegetable consumption by both the elderly and young populations were generally lower than their recommended oral reference doses. The non-carcinogenic risks obtained for both populations were less than one. However, the consumers of *T. triangulare* and *T. occidentalis* from the studied locations were exposed to Pb toxicity, with children being more vulnerable. This study has shown that the application of untreated animal wastes and inorganic fertilizers as manures has the potential to affect human health, bioavailability of metals, and the geochemical fractions of metals in soil over time.

***Keywords*:** soil pollution, *Talinum triangulare*, *Telfairia occidentalis*, fertilizer, transfer factor, Akwa Ibom State

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1. Introduction

Anthropogenic activities have significant impacts on the accumulation and bioavailability of metals in soil and vegetables. Consequently, their levels are usually higher than their recommended limits in our environment. The applications of herbicides, pesticides, and fertilizers by farmers have raised the levels of metals in soil and vegetables [1-4]. Studies have shown that growth promoters given to farm animals have the potential to increase the levels of metals in soil when wastes from animal farms are applied as manures [5, 6]. Although a lot of the elements associated with these growth promoters are needed by both plants and animals, they are poisonous at higher concentrations [7]. It has also been reported that arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are hazardous even at their very low levels [8, 9]. Previous research by Aladesanmi et al. [10] and Onuoha [11] revealed that the application of waste materials from fish ponds in a farm may result in the excessive accumulation of metals in the environment. In this study, metals associated with animal wastes and inorganic fertilizers and having relatively high densities when compared to that of water were carefully selected since their weight is closely related to their toxicities [12].

A report by the United States Environmental Protection Agency (USEPA) [13] indicated that the utilization of organic wastes from refuse dumps as manure can accelerate the availability of toxic metals in soil. Recent studies have also shown that vegetables have the capacity to absorb high levels of metals from polluted environments into their edible parts [14-16]. Hence, the consumption of vegetables from polluted environments is a major route of transferring metals into the human body [17, 18]. Studies have also shown that a polluted soil environment has the potential to accumulate high levels of toxic polycyclic aromatic hydrocarbons (PAHs) and transfer them into the food chain [19-23].

Based on the investigation by Orhuamen et al. [19], *Talinum triangulare* and *Telfairia occidentalis* are widely consumed in the region under investigation. Consequently, the use of untreated animal wastes and inorganic fertilizers for the cultivation of these vegetables may result in the risks associated with metal toxicities being exposed to the consumers. Thus, this practice should be monitored and controlled effectively to forestall disaster in the area. Accordingly, assessing the agricultural soils to determine their toxic metal content is one of the most effective tools for monitoring and averting these unforeseen problems. The actual status of metals in soil with regards to their toxicity can only be ascertained by the speciation study and not by their total concentrations [24, 25]. Reports have also identified a multivariate method for the proper understanding of metal contamination in soil [26-28].

This study evaluated the impact of inorganic fertilizers and manures originating from fish ponds, cattle, pig, and poultry farms on the bioaccumulation, speciation, and health risks associated with toxic metals in soil and vegetables. Basically, this study shall provide information on the intensive use of animal wastes and inorganic fertilizers as manures on the environment and food chain, which has hitherto been scanty in the study area. Multivariate analyses were also carried out to ascertain the actual source and relationship of the toxic elements in the studied locations. The sequential extraction of these toxic elements helps identify their different geochemical forms in soil from the studied locations. The health problems linked to human exposure to *T. triangulare* and *T. occidentalis* cultivated in soils contaminated with organic and inorganic manures shall also be disclosed using standard models. The health problems linked to human exposure to *T. triangulare* and *T. occidentalis* cultivated in soils contaminated with organic and inorganic manures shall also be disclosed using standard models.

2. Materials and methods

2.1 *Study area*

The study is located within the Niger Delta Area at 4° 32’ N and 5° 33’ N (latitudes) and 7° 25’ E and 8° 25’ E (longitudes) (Figure 1). Akwa Ibom State is known as a region with dry and rainy seasons from November to March and April to October, respectively. The weather has had a long period of rain and elevated temperatures. According to Afangideh et al. [29], the annual temperature ranges from 25 °C to 29 °C, while the average yearly rainfall ranges from 2,000 mm to 3,000 mm. These climatic conditions favor intensive agricultural activities in the study area. According to standard soil classifications, the soil type belongs to the Anthrosol class [30]. Table 1 shows the coordinates and sources of contaminants for the studied soils.

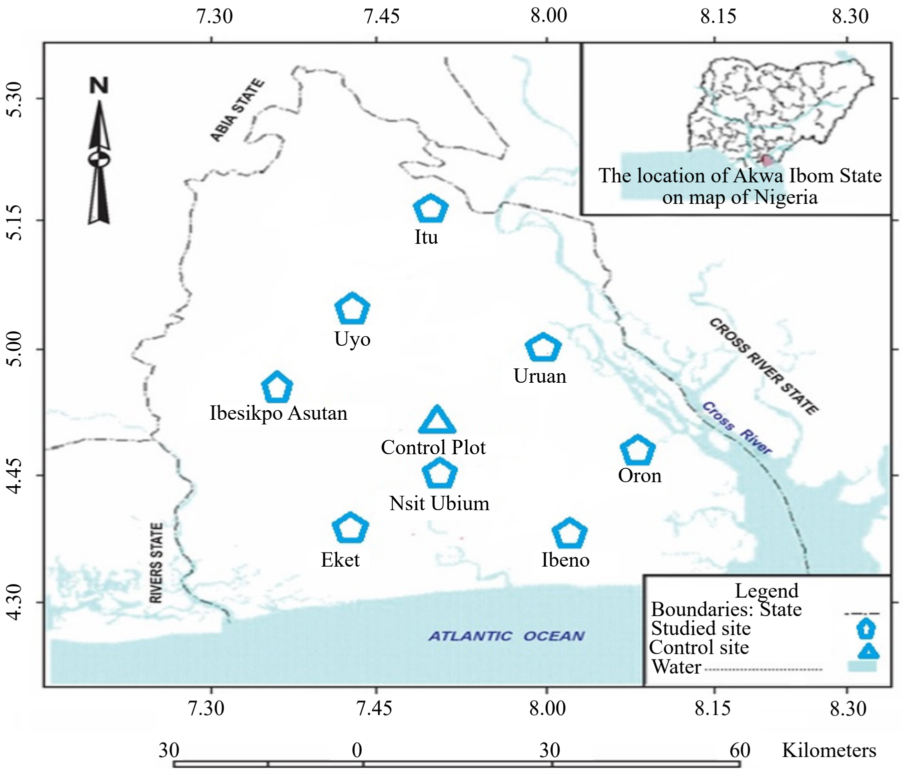


Figure 1. Studied and control sites

Table 1. Locations, coordinates and sources of contaminants

Location	Coordinates	Sources of contaminants
Ibeno	7° 48’ E and 4° 63’ N	Poultry wastes and cow dungs
Itu	7° 59’ E and 5° 09’ N	Poultry wastes
Oron	8° 14’ E and 4° 50’ N	Wastewater from fish ponds and poultry wastes
Nsit Ubium	7° 56’ E and 4° 47’ N	Nitrogen, phosphorus, and potassium fertilizer
Uyo	7° 56’ E and 5° 03’ N	Wastes from dumpsite and wastewater from fish pond
Uruan	8° 05’ E and 5° 04’ N	Inorganic fertilizers and poultry wastes
Ibesikpo Asutan	7° 57’ E and 4° 45’ N	Poultry and piggery wastes
Eket	7° 56’ E and 4° 41’ N	Poultry wastes and wastewater from fish pond
Nsit Ibom (control site)	7° 54’ E and 4° 53’ N	No manure applied

2.2 *Sample pretreatment and analysis*

Soil samples were collected from three points on each of the designated farms at a depth of 0 cm to 15 cm using a soil auger and put together to form a composite sample. *T. triangulare* and *T. occidentalis*, which are widely consumed in Akwa Ibom State, were also collected from these farms using a stainless-steel knife. As a control site, the topsoil and vegetables were obtained from a farm where no manure was applied. Soil samples from the studied locations were dried under the sun for 72 hours, then homogenized and sieved. One gram of the dried soil sample was placed on a hot plate and a 3:1 mixture of HCl and HNO_3 was added to digest the sample completely. The concentration of the metals was later determined using an emission spectrophotometer.

The vegetables studied were washed first with tap water and then later with distilled water. They were air dried, cut into tiny pieces and dried again at 60 ºC for 24 hours in an oven. The dried samples were ground and homogenized using a blender into powdery form. One gram of the ground vegetable was completely digested with a 3:1 mixture of HCl and HNO_3 on a hot plate. The concentrations of metals analyzed in the samples were obtained using an inductively coupled plasma optical emission spectrometer (ICP-OES).

2.3 *Authentication of procedures and results obtained*

The Montana 1 soil, also referred to as SRM 2710a (reference material), was employed for the substantiation of the procedures and the results. SRM 2710a soil was treated and analyzed with similar procedures used for the studied soils. The results obtained are similar to the results certified (See Table 2).

Table 2. Results of metals in the reference material and in soils from the studied locations [31]

Parameter	Standard result ± SD*	Determined mean result ± SD*	Value recovered (%)
As (mg kg^{-1})	1,540.0 ± 100	1,462 ± 0.48	92.6
Cd (mg kg^{-1})	12.3 ± 0.3	10.54 ± 0.42	85.7
Ni (mg kg^{-1})	8.0 ± 1.0	7.31 ± 0.73	91.4
Pb (mg kg^{-1})	5,520 ± 0.003	5,260 ± 0.09	95.3

*SD = standard deviation

2.4 *Toxic metals speciation by optimized Community Bureau of Reference (BCR) method*

The modified BCR procedures of the metal extraction method were employed for the separation of metals into their different geochemical forms [32].

The rate of metals recovered was determined using Equation 1:

$$\text{Percentage of recovery} = \frac{\sum_n \text{BCR extraction method}}{\text{Digestion using a 3:1 mixture of HCl and HNO}_3} \times 100 \tag{1}$$

where n refers to the level of each metal obtained from sequential extraction, while digestion using a 3:1 mixture of HCl and HNO_3 signifies the result of the total concentration of the metal [33].

2.5 *Metal contamination in the soils of studied locations*

2.5.1 *Contamination factor*

Equation 2 illustrates the method used to determine the extent of soil contamination by toxic metals at the studied locations.

$$CF = \frac{Cm}{Bm} \tag{2}$$

In Equation 2, CF, Cm, and Bm represent the contamination factor, metal concentration in the studied site, and metal concentration in the control site, respectively. The contamination factor is classified as either low contamination ($CF < 1$), moderate contamination ($1 \leq CF \leq 3$), considerable contamination ($3 \leq CF \leq 6$), or very high contamination ($CF > 6$) [34].

2.5.2 *Site-to-site contamination level*

Equation 3 was used to calculate the degree of contamination (C_{deg}), which represents the sum of all the contamination factors of toxic metals in a particular soil.

$$C_{deg} = \sum\left(\frac{Cm}{Bm}\right) \tag{3}$$

According to Hakanson [35], C_{deg} can be categorized as either a low degree of contamination ($C_{deg} < 8$), a moderate degree of contamination ($8 < C_{deg} < 16$), a considerable degree of contamination ($16 < C_{deg} < 32$), or a very high degree of contamination ($C_{deg} > 32$).

2.5.3 *Metal transfer rate from soil to vegetable*

The rate of metal transfer from soil to vegetables, referred to as the transfer factor (TF), was evaluated using Equation 4 as described by Taha et al. [36].

$$TF = \frac{Mp}{Ms} \tag{4}$$

In Equation 4, Mp stands for the average metal level in the plant, while Ms represents the metal concentration in the studied soil.

2.6 *Health risk assessment*

This study evaluated the daily intake rate of metals (DI), non-carcinogenic risk (HQ), and chronic hazard index (THI) to ascertain the likelihood of health problems related to the consumption of the studied vegetables.

2.6.1 *Daily intake rate of toxic metals*

To evaluate non-cancer concerns in humans, the risk associated with the metals ingested via *T. triangulare* and *T. occidentalis* was evaluated. The DI was estimated using Equation 5 by following the methods of the USEPA [37] and the human exposure to soil pollutant (HESP) model [38].

$$DI = \frac{C \times Ing\ R \times EF \times ED}{BW \times AT} \tag{5}$$

where C refers to the average metal level, $Ing\ R$ is the ingestion rate of *T. triangulare* and *T. occidentalis*, EF denotes the rate of exposure per day per year, and ED is the annual period of exposure. Meanwhile, BW is the body mass measured in kg and AT refers to the mean period for the non-cancer causing agents [37, 39]. The data for these parameters is shown in Section 2.5.2.

2.6.2 *Non-carcinogenic risk*

Equation 6 was utilized to approximate the non-cancer producing agents of the toxic components based on the risk quotient.

$$HQ = \frac{DI}{Rfd} \tag{6}$$

where Rfd represents the chronic reference quantity of the metals. The reference dose values (mg kg^{-1} day^{-1}) of As, Cd, Pb, and Ni are 0.003, 0.001, 0.0035, and 0.02, respectively [40].

2.6.3 *Overall chronic hazard index*

The totality of the different *HQ* reported in the form of *THI* was estimated using Equation 7.

$$THI = \sum HQ = HQAs + HQCd + HQNi + HQPb \tag{7}$$

The parameters and respective values used were ingestion rate (*IR*; 100 mg/day and 50 mg/day for children and adults, respectively) [39, 41], rate of exposure (350 days/year) [42], period of contact (6 years for children and 30 years for adults) [39], period for the non-cancer causing agents (365 days/year) [43], and mass of the body (15 kg for a child, and 70 kg for an adult) [37, 43].

2.7 *Data analysis*

The results were analyzed using the international business machines-statistical package for the social sciences (IBM-SPSS) Statistics 20 (IBM USA). Factor and cluster analyses were carried out using Duncan's multiple range tests. The Varimax rotation method was used for the factor analysis of the metals, and values ranging from 0.608 and above were applied for the calculation. During this study, dendrograms were utilized for the cluster analysis of similar metal groups.

3. Results and discussion

3.1 *Distribution of toxic metals in the soils of studied and control sites*

The results of toxic metals in soils from the studied locations and the control site are illustrated in Table 3. Table 3 shows a high level of variability between locations. Concentrations of total As ranged from 0.78 mg kg^{-1} to 2.35 mg kg^{-1} in the studied soils. The highest concentration of total As was obtained in Uyo, while the lowest was at Ibesikpo Asutan. Thus, the application of organic wastes from dumpsites and wastewater might considerably affect the As content of the soil in Uyo. This range is higher than the 0.55 mg kg^{-1} to 0.66 mg kg^{-1} reported by Opaluwa et al. [44], but less than the 1.15 mg kg^{-1} to 3.14 mg kg^{-1} obtained by Yahaya et al. [45]. The mean value of As obtained (1.64 ± 0.70 mg kg^{-1}) is lower than the 20.0 mg kg^{-1} recommended by the Food and Agricultural Organization/World Health Organization (FAO/WHO) [46]. Hence, although As may not pose a serious threat in the areas under investigation, its accumulation along the food chain should be monitored.

Table 3. Total concentration of potentially toxic elements in soil samples from the studied locations

Location	As (mg kg^{-1})	Cd (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})
Ibeno	2.35	4.26	67.85	189.32
Itu	1.21	3.87	51.67	175.53
Orin	1.17	3.53	60.97	160.53
Nsit Ubium	2.16	2.36	52.41	166.98
Uyo	2.24	5.55	76.35	217.56
Uruan	0.86	1.36	61.74	226.29
Ibesikpo Asutan	0.78	1.26	56.24	148.19
Eket	2.34	3.15	70.53	218.15
Control site	0.02	0.61	5.32	6.22
Maximum	2.35	5.55	76.35	226.29
Mean	1.64	3.17	62.22	187.83
Standard deviation	0.70	1.47	8.83	29.73
Maximum acceptable limit by FAO/WHO [46]	20.00	3.00	50.00	50.00

Total Cd in the studied soils ranged from 1.26 mg kg^{-1} to 5.55 mg kg^{-1}. This range is higher than the 0.02 mg kg^{-1} to 0.06 mg kg^{-1} reported by Adagunodo et al. [47], but less than the 7.15 mg kg^{-1} to 12.00 mg kg^{-1} obtained by Udosen et al. [48]. The highest level of Cd was also recorded in samples from Uyo, while samples from Ibesikpo Asutan showed the lowest concentration. This study revealed that organic wastes from waste dumps and wastewater utilized as organic manure on the studied soil in Uyo may have increased the level of Cd in the environment. The mean Cd concentration reported in Table 3 exceeds the 3.00 mg kg^{-1} FAO/WHO recommendation for a non-polluted soil [46]. Hence, Cd may be considered a contaminant in the examined areas and may cause substantial health hazards due to its toxicity. Meanwhile, total Ni in the studied locations varied between 51.67 mg kg^{-1} and 76.35 mg kg^{-1}. This is higher than the 0.01 mg kg^{-1} and 0.91 mg kg^{-1} obtained by Ahaneku and Sadiq [49], but lower than the 5.00 mg kg^{-1} to 140.50 mg kg^{-1} reported by Ajiboso et al. [50]. The highest and lowest concentrations of Ni were reported in Uyo and Itu, respectively. This result also showed that dumpsites and wastewater contain high levels of Ni, as confirmed in the samples from Uyo. A mean concentration of 62.22 ± 8.83 mg kg^{-1} was reported for Ni, which is higher than the 50.0 mg kg^{-1} limit for unpolluted soil by FAO/WHO [46]. Thus, the manures applied to the examined soils may have had a major impact on Ni buildup in the area and may have contributed to Ni toxicity issues.

Table 3 shows that the total Pb concentrations reported for studied soils ranged from 148.19 mg kg^{-1} to 226.29 mg kg^{-1}. These values are higher than 0.00 mg kg^{-1} to 125.95 mg kg^{-1} obtained by Olabanji et al. [51] but lower than 259.1 mg kg^{-1} to 735.7 mg kg^{-1} reported by Udousoro et al. [52]. The highest and lowest levels of Pb were reported in the samples from Uruan and Ibesikpo Asutan, respectively. The high total concentration of total Pb at Uruan could be attributed to the intensive applications of inorganic fertilizers [3, 53]. The results in Table 3 also indicate that the mean value of Pb obtained is higher than the 50.0 mg kg^{-1} recommended for soil by FAO/WHO [46]. Hence, the applications of manures may have resulted in a significant accumulation of Pb in the studied soils, which could also cause problems related to a high concentration of Pb.

Generally, the results obtained revealed the impact of excessive applications of manures on the accumulation of these toxic metals in the soils investigated [54-56]. The total concentration of the metals varied as follows: Pb > Ni > Cd > As, with samples indicating very high concentrations of these metals.

3.2 *Contamination factor of toxic metals and the extent of site-to-site contamination of studied locations*

These models were used to evaluate the degree of soil contamination and the degree of site-to-site contamination, respectively [57]. Based on the classifications by Pekey et al. [34], As and Ni belong to the low contamination class, while Cd and Pb are in the very high contamination class. Consequently, the studied soils were high in Cd and Pb but fairly enriched with As and Ni. Thus, the applications of organic wastes and inorganic fertilizers may have elevated the levels of Cd and Pb in the studied soils. The contamination factor of the studied soils followed the trend of Cd > Pb > As > Ni.

The degree of soil contamination shown in Table 4 varies from 12.50 in Ibesikpo Asutan to 30.67 in Uyo. Results showed that studied locations were in the substantial contamination class [35]. Accordingly, the studied locations were severely contaminated with these toxic metals. The rate of site-to-site contamination followed the trend of Uyo > Ibeno > Eket > Itu > Oron > Nsit Ubium > Uruan > Ibesikpo Asutan. Thus, organic wastes from dumpsites and wastewater applied to the Uyo soil might have significantly increased the concentrations of these toxic metals in the area.

Table 4. Contamination factor of potential toxic elements and the extent of site-to-site contamination (C_{deg}) of studied soils

Location	As	Cd	Ni	Pb	C_{deg}
Ibeno	0.18	14.18	1.00	9.47	24.83
Itu	0.09	12.91	0.76	8.78	22.54
Orin	0.09	11.75	0.90	8.03	20.77
Nsit Ubium	0.17	7.88	0.77	8.35	17.17
Uyo	0.17	18.50	1.12	10.88	30.67
Uruan	0.07	4.53	0.91	11.31	16.82
Ibesikpo Asutan	0.06	4.20	0.83	7.41	12.50
Eket	0.18	10.50	1.04	10.91	22.63
Minimum	0.06	4.20	0.76	7.41	12.50
Maximum	0.18	18.50	1.12	11.31	30.67
Mean	0.13	10.56	0.92	9.39	20.99

3.3 *Metal speciation results from soils of studied and control sites*

Table 5 shows the results for metal speciation in soils from studied agricultural soils and control sites. As existed predominantly in the reducible fraction. This is consistent with reports by Khan et al. [58] and Ebong et al. [59]. The fraction contributed a total of 61.0% and 61.5% in soils from the studied and control sites, respectively. The readily available, oxidizable, and residual fractions contributed a total of 39.0% and 38.5% for soils from the studied and control sites, respectively. This shows human inputs of As in the environment, as reported by Farkas et al. [60]. The speciation of As in both areas (studied and control sites) followed the trend of reducible (RED) > residual (RES) > acid extractable (AEX) > oxidizable (OX).

Table 5. Mean speciation, total metal, and percentage of recovery of toxic metals in soils from studied and control sites

	As (mg kg^{-1})	Cd (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})
		Studied soils		
AEX	1.376	13.945	90.137	336.073
RED	7.342	5.617	121.80	714.538
OX	1.018	2.534	54.175	215.327
RES	2.281	1.840	217.625	143.640
TF	12.017	23.936	483.743	1409.578
TM	13.117	25.336	497.747	1502.539
%REC	91.6	94.5	97.2	93.8
		Control soils		
AEX	0.002	0.304	0.717	1.208
RED	0.008	0.113	0.746	2.865
OX	0.001	0.102	0.625	1.143
RES	0.002	0.061	2.832	0.639
TF	0.013	0.580	4.920	5.855
TM	0.015	0.613	5.324	6.217
%REC	86.6	94.6	92.4	94.2

Note: AEX = readily available; RED = oxides and hydroxides of Fe and Mn; OX = fraction associated with organic matter and sulphur; RES = inert; TF = sum of all fractions; TM = total metal; and %REC = percentage of recovery

Cd occurred mainly in the readily available fraction both in soils from the studied and control sites (Table 5). This agrees with the research by Kashem et al. [61] and Osakwe [62]. The acid extractable fraction of Cd contributed a total of 58.2% and 52.4% of the soils from the studied and control sites, respectively. Three other fractions (RED, OX, and RES) contributed a total of 41.7% and 47.6% of the soils from the studied and control sites, respectively. Higher Cd concentrations in the readily available part of soils from study sites may have contributed to the high contamination factor shown in Table 4. The speciation of Cd in soils from examined locations and the control site varied as follows: AEX > RED > OX > RES. This trend should be controlled to prevent the environmental issues associated with excessive Cd levels.

Results in Table 5 indicate that Ni existed mainly in non-available form (RES) in the studied agricultural soils and control plot. The result is similar to that obtained by Kotoky et al. [63] and Ebong et al. [64]. The residual fraction of Ni contributed a total of 45.0% and 57.5%, respectively, in soils from the studied and control sites. The other three segments donated a total of 55.0% and 42.5% of the studied agricultural soils and the control site, respectively. This study showed that Ni was not readily available in the study area as previously reported [65]. This might have resulted in the low contamination factors recorded for Ni in Table 4. The speciation of Ni varied as follows: RES > RED > AEX > OX.

Pb in the studied and control sites were associated mostly with oxides and hydroxides of Fe and Mn (Table 5). The result agrees with that reported by Ebong et al. [59] and Rodgers et al. [66]. This shows the introduction of Pb through human activities to the areas investigated [60]. The reducible fraction of lead in both soils from the studied areas and the control plot contributed 50.7% and 48.9%, respectively, to the total fraction. Consequently, the other three fractions (acid extractable, oxidisable, and residual) of Pb in soils from the studied locations and control plot contributed 49.3% and 51.1%, respectively, to the total fractions. Variability in the speciation of Pb in both soils from studied locations and the control plot was as follows: RED > AEX > OX > RES.

The high percentage of recovery of the metals reported in Table 5 for soils from studied and control sites indicates a high degree of accuracy in the procedures used and the reliability of the results obtained. Based on the report by Ahumuda et al. [67], the organic and inorganic manures applied to the studied soils might have affected these metals differently, thereby resulting in variations in their speciation results between the studied and control sites.

3.4 *Toxic elements levels of vegetables from studied and control sites*

Table 6 shows the total concentration of the metals in *T. triangulare* and *T. occidentalis*. As ranged from 0.05 mg kg^{-1} to 0.12 mg kg^{-1} in *T. triangulare* and 0.04 mg kg^{-1} to 0.10 mg kg^{-1} in *T. occidentalis*. These values are lower than the 0.03 mg kg^{-1} to 1.15 mg kg^{-1} reported by Okorosaye-Orubite and Igwe [68], but greater than the 0.016 mg kg^{-1} to 0.065 mg kg^{-1} recorded by Abbas et al. [69]. The highest level of As was obtained in *T. triangulare* from Uyo, while the lowest was in *T. occidentalis* from Ibeno. The levels of As in the studied vegetables were higher than those obtained in their control sites. This indicates the negative impact of organic and inorganic manures on the bioaccumulation of As in these vegetables [70]. *T. triangulare* showed a higher tendency to accumulate As from the studied soils than *T. occidentalis* [71]. The mean levels of As in both vegetables exceed the 0.43 mg kg^{-1} FAO/WHO recommendation for vegetables [46]. Consequently, the consumption of these vegetables could result in health problems related to high As [71].

Table 6. Total concentration of trace metals in *T. triangulare* and *T. occidentalis*

Location		As (mg kg^{-1})	Cd (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})
Ibeno	*T. triangulare*	0.05	0.09	2.83	4.794
	T. occidentalis	0.04	0.10	2.43	1.7
Itu	*T. triangulare*	0.07	0.10	2.16	3.54
	T. occidentalis	0.04	1.12	1.05	1.01
Oron	*T. triangulare*	0.07	0.10	3.54	2.18
	T. occidentalis	0.05	0.09	1.26	0.86
Nsit Ubium	*T. triangulare*	0.10	0.07	2.73	3.79
	T. occidentalis	0.06	0.16	2.66	1.59
Uyo	*T. triangulare*	0.12	0.09	3.52	5.06
	T. occidentalis	0.06	0.08	2.47	2.14
Uruan	*T. triangulare*	0.06	0.09	1.21	2.56
	T. occidentalis	0.08	0.10	0.86	1.10
Ibesikpo Asutan	*T. triangulare*	0.05	0.08	1.25	3.54
	T. occidentalis	0.07	0.07	0.75	1.47
Eket	*T. triangulare*	0.08	0.08	3.13	5.27
	T. occidentalis	0.10	0.06	2.09	1.68
Control site	*T. triangulare*	0.001	0.05	0.08	0.07
	T. occidentalis	0.002	0.01	0.05	0.04
Minimum	*T. triangulare*	0.05	0.07	1.21	2.18
	T. occidentalis	0.04	0.06	0.75	0.86
Maximum	*T. triangulare*	0.12	0.10	3.54	5.27
	T. occidentalis	0.10	0.16	2.66	2.14
Mean	*T. triangulare*	0.07	0.09	2.55	3.84
	T. occidentalis	0.06	0.10	1.70	1.45
Standard deviation	*T. triangulare*	0.03	0.01	0.93	1.14
	T. occidentalis	0.02	0.03	0.80	0.43

The levels of total Cd ranged from 0.07 mg kg^{-1} to 0.10 mg kg^{-1} in *T. triangulare* and 0.06 mg kg^{-1} to 0.16 mg kg^{-1} in *T. occidentalis*. The general range is lower than the 0.07 mg kg^{-1} to 0.97 mg kg^{-1} as obtained by Kananke et al. [72], but higher than the 0.017 mg kg^{-1} to 0.039 mg kg^{-1} as reported by Malum and Salihu [73]. The highest level of Cd was obtained in *T. occidentalis* from Nsit Ubium, while the lowest level was reported for *T. occidentalis* from Eket. *T. occidentalis* indicated a higher capacity to accumulate Cd from the studied soils than *T. triangulare*. The mean values of Cd in the studied vegetables were higher than those at the control site (Table 6). As a result, the application of inorganic fertilizer in the studied Nsit Ubium soil might have affected the level of Cd in the vegetables. The mean values of Cd in both types of vegetables are lower than the recommended limit of 0.20 mg kg^{-1} by FAO/WHO [46].

As shown in Table 6, Ni concentrations in *T. triangulare* and *T. occidentalis* range between 1.21 mg kg^{-1} and 3.54 mg kg^{-1} and 0.75 mg kg^{-1} and 2.66 mg kg^{-1}, respectively. This is consistent with the range of 0.21 mg kg^{-1} to 3.54 mg kg^{-1} reported by Akan et al. [74] but lower than the 0.54 mg kg^{-1} to 10.11 mg kg^{-1} obtained by Likuku and Obuseng [75]. The highest level of Ni in *T. triangulare* was reported in Oron, while the lowest level was obtained in *T. occidentalis* from Ibesikpo Asutan. *T. triangulare* showed higher potential for accumulating Ni from soil than *T. occidentalis*. The application of poultry waste and wastewater from fish ponds at Oron might have resulted in the high level of Ni in these vegetables [4, 76]. The Ni levels obtained for the studied vegetables were much higher than values reported for those from the background soil. However, the levels of Ni in *T. triangulare* and *T. occidentalis* are lower than the 4.00

mg kg^{-1} recommended limit according to FAO/WHO [46]. Thus, human exposure to the vegetables might not result in serious health problems related to Ni toxicity.

The levels of Pb varied from 2.18 mg kg^{-1} to 5.27 mg kg^{-1} in *T. triangulare* and from 0.84 mg kg^{-1} to 2.14 mg kg^{-1} in *T. occidentalis*, respectively. The general range obtained is greater than the 0.25 mg kg^{-1} to 4.56 mg kg^{-1} reported by Akan et al. [74], but lower than the 1.20 mg kg^{-1} to 17.18 mg kg^{-1} obtained by Lawal et al. [77]. The highest Pb level was recorded in *T. triangulare* from Eket, while the lowest was reported in *T. occidentalis* from Oron. This indicates that a substantial quantity of anthropogenic Pb was added to the soil at Oron by the application of wastes from fish ponds and poultry farms and subsequently transferred to these vegetables [4]. The mean values of Pb in the studied vegetables were higher than the values in vegetables from the control site (Table 6). Relatively, *T. triangulare* has a higher tendency to accumulate Pb from the studied soils than *T. occidentalis*. The mean values of Pb in *T. triangulare* and *T. occidentalis* are higher than the 0.30 mg kg^{-1} recommended by FAO/WHO [46]. Consequently, exposure to these vegetables might result in serious human health problems, as indicated by the Agency for Toxic Substances and Disease Registry (ATSDR) [78].

In general, the amounts of metals in the investigated vegetables were significantly greater than those measured in the background soil. The application of organic and inorganic manures may have led to the bioaccumulation of these metals in the investigated locations. Consequently, the use of organic and inorganic manures on the farm has the potential to impact the quality of the soil and the plants cultivated there.

3.5 *Transfer factors of toxic metals from soil to vegetables*

The quantity of these toxic metals transferred from the studied soils into *T. triangulare* and *T. occidentalis* and the level of human exposure to these metals were also assessed using the transfer factor [79, 80]. Hence, the transfer factor indicates the level of human exposure to these toxic metals through the consumption of the studied vegetables. Results for the transfer factors of metals in *T. triangulare* and *T. occidentalis* are illustrated in Figure 2. The transfer factors of As, Cd, Ni, and Pb in the studied vegetables are 0.02 to 0.85, 0.01 to 0.64, 0.01 to 0.06, and 0.01 to 0.03, respectively. As shown in Figure 2, the transfer factors of metals are generally less than one, as earlier reported by Puschenreiter and Horak [81]. According to Bahemuka and Mubofu [82], these vegetables could be used as excluders and for phytostabilization but not for phytoextraction of metals. The low transfer factors identified may be associated with a low level of consumer exposure to metals through the consumption of these vegetables [83].

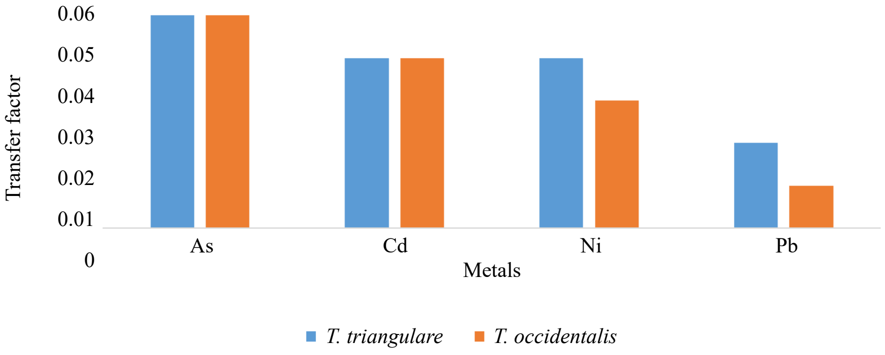


Figure 2. Mean level of metals transferred from soil to vegetables

3.6 *Health risk assessment*

3.6.1 *Results for the daily intake rate of toxic metals*

The ratio of daily metal intake by both the young and old populations through the consumption of *T. triangulare* and *T. occidentalis* is shown in Table 7. Human exposure to harmful metals and the associated effects are fundamentally assessed by their daily intake rate [84]. Table 7 indicates that the *DI* of metals in the vegetables for both populations was lower than their *Rfd* values [40]. Thus, exposure to these vegetables may not cause serious human health problems, but their bioaccumulation in this biota should be avoided. The study revealed higher rates of daily intake for the young than the elderly population, as previously reported by Song et al. [85]. Results in Table 7 indicate the order of the *DI* of both populations as Pb > Ni > Cd > As. The results also showed that the consumers are more exposed to Pb through the consumption of *T. triangulare* while they are more exposed to Cd, Ni, and As via consumption of *T. occidentalis*. In all cases, the young population is the most vulnerable class. The *DI* values for metals via the consumption of *T. occidentalis* followed the order of Ni > Pb > Cd > As. The low *DI* values reported should not be taken for granted, as these metals are harmful even at their low concentrations [86].

Table 7. Non-carcinogenic hazard of metals and their exposure routes via *T. triangulare* and *T. occidentalis* consumption

	T. triangulare				*T. occidentalis*			
	DI		*HQ*		*DI*		*HQ*	
Metal	Young	Old	Young	Old	Young	Old	Young	Old
As	5.7E-6	3.03E-6	0.0019	0.001	4.8E-6	2.5E-6	0.002	0.0008
Cd	6.5E-6	3.40E-6	0.0065	0.0034	7.4E-6	3.9E-6	0.007	0.0039
Ni	1.9E-4	1.04E-4	0.0100	0.005	1.3E-4	6.9E-5	0.007	0.0030
Pb	2.9E-4	1.57E-4	0.0730	0.039	1.1E-4	5.9E-5	0.030	0.0150
THI			0.0914	0.0484			0.046	0.0227

3.6.2 *Results for the non-carcinogenic risk*

The *HQ* of metals in *T. triangulare* and *T. occidentalis* is shown in Table 7. The *HQ* of the elements in the studied vegetables was less than one. Hence, these metals may not have the potential to cause serious harm to either the young or the elderly populations. The *HQs* of metals for both populations via the consumption of *T. triangulare* varied as follows: Pb > Ni > Cd > As. However, the *HQ* values for the metals via *T. occidentalis*' consumption varied as Pb > Cd = Ni > As for children and Pb > Cd > Ni > As for the elderly. The consumption of these vegetables by both populations could be affected by Pb, with the child population being more exposed. Based on the report by Man et al. [87], both populations consuming these vegetables might be exposed to non-carcinogenic risks even though their *THI* values are lower than one.

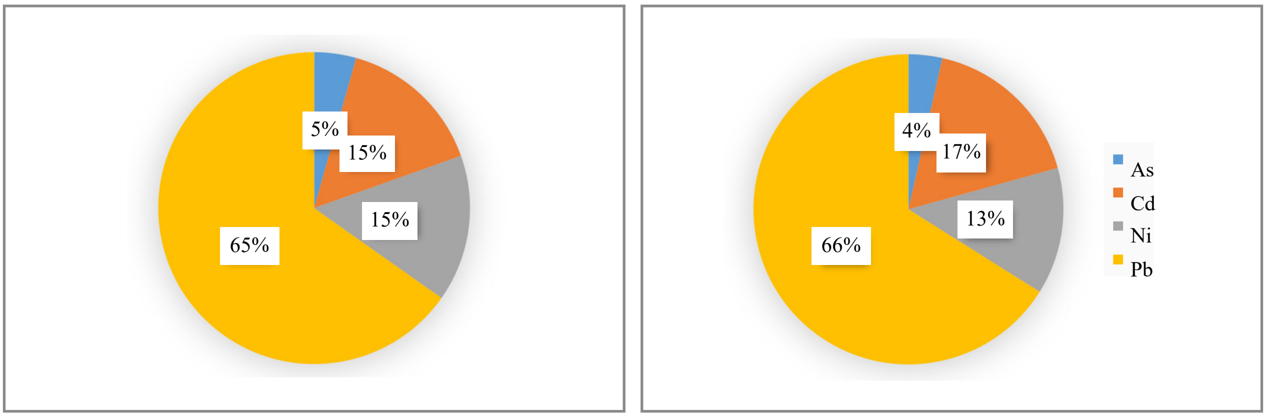
3.6.3 *Results for the overall chronic risk index*

Table 7 presents the results for *THI* values of oral contact with these toxic metals for both young and elderly individuals through exposure to the examined vegetables. As, Cd, Ni, and Pb *THI* values for the young and elderly populations are 0.0914 and 0.0484, respectively. The study revealed a higher *THI* value for the young population than for the elderly. Thus, the young population is more prone to health issues.

a b

Figure 3. Mean risk index for (a) young and (b) old population through exposure to *T. triangulare*

The *HQ* results revealed that the young and elderly populations contributed a total of 80% and 81% of Pb, respectively (Figures 3(a) and (b)). Even if the *THI* value is below one, both populations consuming *T. triangulare* are more exposed to high levels of Pb and its associated dangers. The overall *HQ* values of the other metals (As, Cd, and Ni) among young and elderly populations via *T. triangulare* consumption contributed 20% and 19%, respectively, to the overall chronic risk index.



a b

Figure 4. Mean risk index for (a) young and (b) old populations through exposure to *T. occidentalis*

Table 7 shows the *THI* values of the metals for the young and old populations through *T. occidentalis'* consumption as 0.046 and 0.0227, respectively. The child population is more disposed to human health-related problems via *T. occidentalis* consumption. Figures 4(a) and (b) indicate that the *HQ* values of Pb among the young and old populations contributed a total of 65% and 66%, respectively, to the risk index. This shows that consumers of *T. occidentalis* are also more exposed to health risks related to Pb toxicity than other metals. The total *HQ* values of As, Cd, and Ni for children and adult populations via the consumption of *T. occidentalis* contributed a total of 35% and 34%, respectively, to the enduring risk index.

3.7 *Multivariate analysis*

Principal component analysis (PCA) was used to evaluate the major factors responsible for the accumulation of metals in soils at the studied sites. Moreover, hierarchical cluster analysis (HCA) was used to analyze the relationship between the metals in soils from the studied locations.

3.7.1 *Results for the overall chronic risk index*

According to the reports by Wu and Kuo [88], PCA is an essential tool used to determine the factors accountable for the accumulation of contaminants or pollutants in an environment. The PCA results in Table 8 indicate one major factor responsible for the buildup of these metals in soils from the studied locations. The identified factor contributed 63.3% of the entire variance and showed significant positive relationships with all the metals determined. This factor could be mainly the applied organic and inorganic manures as earlier observed [89-91].

Table 8. Total variance of toxic metals determined in soils from the studied locations

Component	Original eigenvalues			Extraction quantities of squared loadings			Component	
	Total	Percentage of variance	Cumulative percentage	Total	Percentage of variance	Cumulative percentage	1	
1	2.532	63.3	63.3	2.532	63.3	63.3	Ni	0.90
2	0.851	21.3	84.6				As	0.80
3	0.401	10.0	94.6				Cd	0.77
4	0.216	5.4	100.0				Pb	0.71

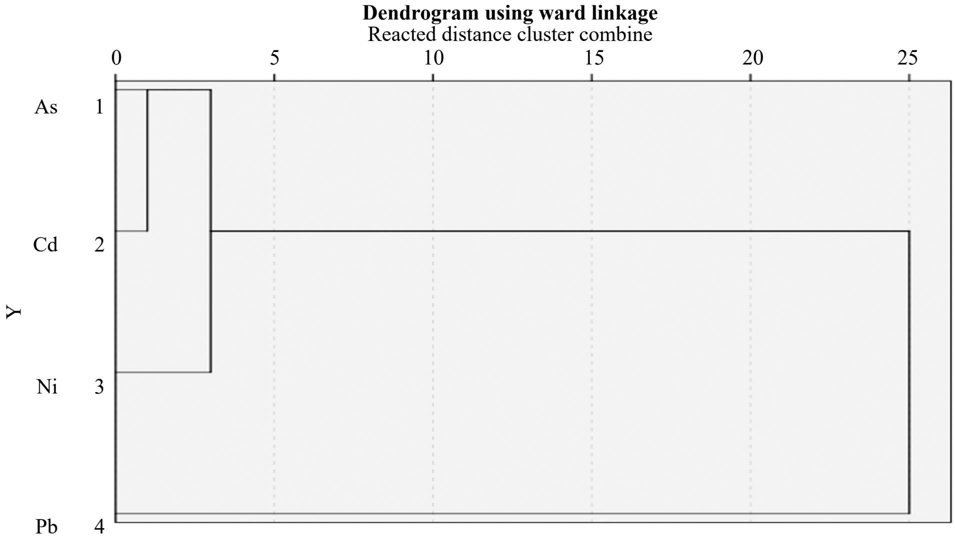


Figure 5. Hierarchical clusters of toxic elements in soils from studied locations

Figure 5 shows the common relationship existing among the metals analyzed in the studied soils. There are three major clusters: the cluster connecting As and Cd, the cluster connecting only Ni, and the cluster connecting only Pb. Accordingly, there could be a very close relationship between As and Cd in the studied soils, while the relationship between Ni and Pb may vary. Studies have shown that As is a common component of the organic and inorganic parts of the soil. Thus, it could be closely related to the organic and inorganic manures in the environment investigated and to the different geochemical fractions reported [92, 93].

4. Conclusion

This study has shown the negative effects linked to the applications of untreated waste materials from animal farms to agricultural soils on the quality of the soil and the vegetables cultivated. It has also revealed the impact of inorganic fertilizers on the bioaccumulation of toxic metals in soil and vegetables in the oil producing area of Nigeria. The results of this research revealed higher levels of toxic metals in soils and vegetables from the studied locations than in the area without organic and inorganic manures (control site). The relatively higher concentrations of these toxic metals in the studied soils as compared to the control site is an indication of the likelihood of these organic wastes and inorganic fertilizers impacting negatively on the environment over time. Results obtained from the studied soils indicated that the mean concentrations of all the metals determined were above their permissible limits except for As. Meanwhile, the mean concentrations of As, Cd, and Pb in the studied vegetables were higher than their recommended limits. The study also revealed that As and Pb were associated mostly with the oxides and hydroxides of Fe and Mn. Cd was in the readily available form, while Ni existed predominantly in the inert fraction. The transfer factor of the metals in the studied vegetables was generally lower than one; hence, these plants were considered excluders. PCA ascertains anthropogenic factor (human activities) as the main route of toxic metal exposure to the soils investigated. The non-carcinogenic risks related to exposure to *T. triangulare* and *T. occidentalis* were generally low. However, consumers were more exposed to Pb toxicity than other metals, with the child population being more susceptible. Consequently, the intensive applications of animal waste and inorganic fertilizers in agricultural soils should be properly managed since there is a tendency to negatively impact on the environment with time.

Conflict of interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this research.

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