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BIOACCESSIBILITY STUDY AND HUMAN RISK ASSESSMENT OF POTENTIALLY TOXIC ELEMENTS IN POLLUTED SOILS FROM LAGOS, NIGERIA

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ABSTRACT

The simplified bioaccessibility extraction test (SBET) which simulates the acid environment of human gastrointestinal tract was used to evaluate the oral bioaccessibility of Potentially Toxic Elements (PTEs) (As, Cd, Cr, Cu, Mn, Ni, Pb and Zn) in soils from selected dumpsites with high human exposure aroundLagos, Nigeria. The soil samples were acid digested using a microwave digestion system. The total and the bioaccessible concentrations of the PTEs were determined using Inductively Coupled Plasma-Mass Spectrometer. The result of bioaccessibility test showed varying concentrations for PTEs studied with values ranging from 0.10 – 1.91 mg/kg As, 0.10 – 7.88 mg/kg Cd, 0.12 – 4.4 mg/kg Cr, 4.50 – 2990 mg/kg Cu, 32.4 – 193 mg/kg Mn, 0.0 – 24.9 mg/kg Ni, 3.30 – 874 mg/kg Pb and 68.2 – 1960 mg/kg for Zn. Amongst the PTEs, Cd and Zn showed higher bioaccessibility (up to 90%) , Pb and Mn had moderate bioaccessibity whilst As, Cr and Ni had the lowest percent bioaccessibility (< 20%).

Key words: Bioaccessibility, dumpsite, gastrointestinal, oral ingestion, potentially toxic elements, soil

INTRODUCTION

The presence of potentially toxic elements in urban soils is a risk for both the environment and human health. In recent years, the attention of researchers has focused on the bioaccumulation of PTEs due to increasing human activities resulting in extensive pollution of urban soils worldwide [1]. The PTEs present significant risks to human health through ingestion of soil inadvertently or directly. Oral ingestion of soil is therefore considered an important pathway for PTEs to enter the human body [2].

PTEs may accumulate in fatty tissues of human bodies, have middle and long-term health risks, and can adversely affect their physiological functions, disrupt the normal functioning of internal organs, or act as cofactors in other diseases [3]. Ingestion of soil may occur either inadvertently due to poor hygiene, consumption of inadequately washed foodstuffs or deliberately due to geophagia and pica behaviour e.g. the hand to mouth behaviour of children [4]. The total concentrations of PTEs in soils have been well studied worldwide, however, it has been observed that determination of total concentrations of potentially harmful elements alone may be an overestimation of the actual health risks associated with them[5]. Consequently, in order to adjust the risk calculations and take into account the fact that only a portion of the ingested soil actually reaches the blood stream of a human, a realistic assessment of actual health risks requires evaluation of the bioaccessible concentrations. According to Jayawardenea et al. [6], metal absorption in humans depends on the transport of metal via the mucosal cells and reaching the target tissue or the blood stream.Hence, it has been proposed that notions of bioavailability and bioaccessibility be included in risk assessments [7].

Many experimental (*in vivo*) methods have been developed to predict oral bioavailability of soil elements. However, each has its limitations leading to the development of *In vitro* bioaccessibility tests which are now increasingly being used. In vitro tests are inexpensive, physiologically-based extraction tests designed to estimate the bioaccessibility of elements along exposure pathways [8]. Numerous difficultieswhich include long extraction times, large amount of unstable reagents, concentrations near or below detection limitshave beenencountered in some of these protocols/tests [9-11].

The development of the Simplified BioaccessibilityExtraction Testwas in response to a request by the USEPA region III and other US agencies, on the need for laboratories to be able to use and apply simple bioaccessibility tests [12].

The SBET method was adapted by the British Geological Survey after considering the apparently good correlation of bioavailability and gastric bioaccessibility and the limited benefit of including an intestinal phase [8, 13]. For instance, some researchers used a fluid that contained pepsin and a mixture of citric, malic, lactic, acetic, and hydrochloric acids. When the bioaccessibility of a series of test substances were compared using 0.4 M glycine buffer (pH 1.5) with and without the inclusion of these enzymes and metabolic acids, no significant difference was observed (p=0.196), in contrast to the other in vitro digestion methods in which the metals can form new complexes with soil or complexes that may precipitate due to the neutral pH environment. This indicated that the simplified buffer employed in the procedure was appropriate, even though it lacked some constituents known to be present in gastric fluid [8, 14].

In the present study, the bioaccessible concentrations of PTEs through oral ingestion of soil using simplified bioaccessibility extraction test was determined with the aim of evaluating the potential health risk associated with the ingested soils. The researchers of this study observed through bibliographic survey that very limited report [14, 15] is available in Nigeria and other African countries on the use of bioaccessibility test as a tool for evaluation of potential health risk associated with PTEs through direct or accidental ingestion of soils. The majority of researches done were based on the determination of total concentration of PTEs rather than the bioaccessible fraction, hence, the need for this study.

EXPERIMENTAL

Instruments and Reagents

The analysis of the total and bioaccessible concentrations of soil extracts was carried out using Agilent model 7700s Inductively Coupled Plasma Mass Spectrometer (USA). Measurements were made under optimized operating conditions of the ICP-MS. The working standard solution for the calibration of the instrument were prepared daily in 2% HNO_3 (v/v) from multi-element standard stock solution (As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) standardstock solution (1003 mg/L) obtained from Qmx Laboratories, Essex, UK with a nominal concentration of 10 mg/L for every analyte of interest.. The total digestion of soil samples was done using CEM MARSXpress microwave digestion system.All reagents used were analytical grade. All glassware and non-glassware were soaked overnight in 10% $HNO_3$and rinsed with deionised water before use.Hydrochloric acid (36.5–38%) and nitric acid (69%) were obtained from Sigma Aldrich(Gillingham, UK). 0.45mm Acrodisc® cellulose acetate membrane syringe filter were purchased from Sigma Aldrich (Gillingham, UK). An end-over-end rotator placed inside an incubator (Stuart® SI500 shaking incubator) manufactured by Barloworld Scientific Ltd., Staffordshire, UK was used for SBET extraction.Glycine purchased from Fisher

Scientific(Loughborough, UK) was used for extraction of bioaccessible PTEs. All prepared solutions were stored at 4 °C until further use.

Study area and sites

Soil samples used in this study comprise of urban soils collected from seven locations around Lagos metropolis, Nigeria. Six of the locations were contaminated sites mostly dumpsites at a distance of 60-100 m from residential and commercial areas while the seventh sample was a control site devoid of anthropogenic activities (Table 1).

Table 1: Description of Sampling location and Anthropogenic Activities on sites

Sampling Location	Sample Identifier	Activities	Coordinates
Iwaya	MFM	Dumpsite near a mechanic shop at 2nd gate University of Lagos.	N 06 30' 42.3" E 003 23' 16.7"
Orile	ORL	Welding, Smelting and sale of metal rods	N 06 31' 13.9" E 003 23' 49.3"
Abule Egba	KATANG	A massive dumpsite for electronic wastes, metal scraps	N 06 38' 43.4" E 003 18' 16.5"
Ikorodu	OWD	Metal scarps, iron rods and battery dumpsite	N 06 44' 09.7" E 003 25' 08.7"
Ibafo	IBF	Dumpsite along the roadside near trailer park	N 06 43' 44.4" E 003 24'57.2 "
Akoka	FSS, University of Lagos	Dumpsite across the Faculty of Social Sciences University of Lagos	N 06 30' 52.3" E 003 23' 32.2"
	CONTROL	Botanical garden, University of Lagos	N 06 31' 32.3" E 003 24' 10.1"

Sample collection and preparation

Fifteen samples of soils were collectedat a depth of 0-10 cm a distance of 5 m apart from each of the sampling points and mixed to obtain composite samples for each site.Collected soil samples were put into clean polythene bags and taken to the laboratory in less than 24 hours. The soil samples were placed in polyethylene containers and taken to the laboratory where they were homogenized, air dried, sieved to remove unnecessary matter and stored for further analysis. The detailed information of each sampling location is given in Table 1.

Digestion of Soils

The soil samples were digested using *aqua regia solution* by placing approximately 1.0 g of oven-dried soil into a 50 mL Teflon vessel pre-cleaned with 5 % concentrated nitric acid. 10 mL of *aqua regia* (HCl: HNO_3, 3: 1 v/v) was added to the soil [1], the mixture gently swirled to homogenize the sample with the reagents and left overnight in order to suppress any violent reaction due to effervescence from the soil solution (which may lead to explosion in the microwave). The Teflon vessels with the solution were properly sealed, introduced into the carousel and placed on the drive lug of the CEM MARSXpress microwave digestion system. All the vessels containing samples were properly arranged prior to starting the microwave digestion process and subsequently subjected to microwave dissolution for 20 mins using operating temperature of 128 ^oC and power of 800W. After digestion, cooling time of 20 mins was allowed while the sample vessels were still in the microwave oven. The resulting solution was filtered afterward into 50 mL volumetric flask using 11 mm whatman filter paper, diluted with deionised water to the mark and stored in a centrifuge tube at 4 $^{\mathbf{o}}$C prior to ICP-MS analysis.

Simplified Bioaccessibility Extraction Test

The test was carried out by addingapproximately 0.5 g of soil sample into 50 mL gastric solution, a synthetic fluid composed of 0.4 M glycine adjusted with 12 M HCl to pH 1.5 ± 0.2in a HDPE screw top tube [10]. The mixture was agitated in an end-over-end orbital shaker maintained at 37 ^oC and 100 rpm for 1 hr. Temperature was maintained at 37°C throughout the extraction process. After extraction, some aliquot was removed with a 0.45 µm cellulose acetate membraneacrodisc® syringe filter pre-cleaned with 0.4 M glycine. For each sample extraction process carried out, pH value of the sample solution was taken at the beginning and the end of the extraction to ensure it was within required range of pH 1.3 -1.7 before filteration otherwise the procedure has to be repeated. The filterates transferred into a 10 mL centrifuge tube and stored in a refrigerator at 4 ^{0} C until ICP-MS analysis within a week. Quality control measure was taken on the discovery that sufficient amount of Zn was found in the cellulose acetate membrane filter used by performing blank correction for all results obtained during ICP-MS analysis [17]. This occurred as a result of the use of Zn as an intrinsic component in the filter manufacture and so could not be removed, but was fortunately overcome by means of blanksubtraction. Triplicate extraction was done and also internal standardization was used for each sample during ICP-MS analysis as quality control measure.The operating conditions of ICP-MS instrument are as summarised in Table 2.

Table 2: Operating conditions of Agilent 7700s ICP-MS

Parameter	Mode
RF Power	1550 W
Carrier gas flow	1.05 L / min
Nebulizer pump	0.1 rps
Quadrupole bias	-15 V
Omega Lens	8.4 V
Reaction cell	ON
Sampling period	0.31 s
Internal standard	^{115}In

RESULTS AND DISCUSSION

Total concentrations of PTEs in Soils

Varying values of PTE concentrations were found for the different soil samples investigated in this study (Fig. 1) with values obtained in the following range; As 0.7– 20 mg/kg, Cd < 0.04 -20 mg/kg, Cr 2.4 – 2410 mg/kg, Cu 14.3– 14900 mg/kg, Mn 131 – 3020 mg/kg, Ni 7.0 – 1050 mg/kg, Pb 17.2 – 6200 mg/kg and Zn 71 – 2760 mg/kg respectively. These values were compared with background and soil guideline values from several countries. Since, as at the time of this study no SGVs existed for Nigeria, it was considered necessary to compare the result obtained in this study with soil background values [18] and more specifically South African SGVs [19] (the only country in the African continent with SGVs). When compared with South African recommended soil guideline values it was observed that the levels of some PTEs such as As, Cd, Cr, and Ni were below the SGVs while other PTEs such as Cu, Mn, Pb and Zn were found to be higher, especially the soils from KATANG, ORL and OWD sites, which may be as a results of the anthropogenic activities on thesoils.However, the results obtained for levels of PTEs from the control site were quite low when compared to the sampled dumpsites.

Bioaccessible concentrations of PTEs in Soils

The levels of PTEs bioacessible to humans through oral ingestion of soil using SBET protocol were found to be relatively low, with values obtained mostly below the USEPA recommended oral ingestion rate for soil [7]especially for Cr, Mn, Ni with the exception of As, Pb, Cu and Zn from KATANG and OWD soils which had levels beyond USEPA recommmended ingestion rate [7]. An indication that very high risk exist for humans in close proximity to KATANG and OWD soils, more importantly children who play with soil around the sites as well as workers or scavengers who usually frequent these dumpsites to pick solid wastes materials to resell to people or companies for recycle. The range of bioaccessible concentrations of PTEs varied widely among the elements as follows: 0.10 – 1.91 mg/kg for As, 0.10 – 7.88 mg/kg for Cd, 0.12 – 4.4 mg/kg for Cr, 4.50 – 2990 mg/kg for Cu, 32.4 – 193 mg/kg for Mn, 0.0 – 24.9 mg/kg for Ni, 3.30 – 874 mg/kg for Pb and 68.2 – 1960 mg/kg for Zn. It was further observed that the soil with the highest bioaccessible concentration of PTEs was KATANG soil, followed by ORL and OWD soils which may be primarily due to their very high total PTE concentrations.

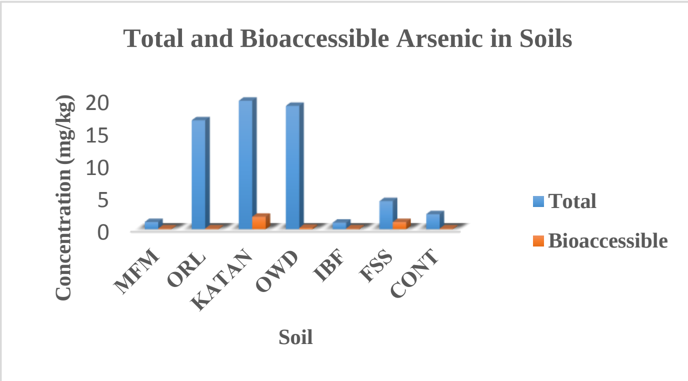
Subsequently, the percent bioaccessibility of each PTE was calculated in order to evaluate the fraction of the PTEs bioaccessible from the total PTE concentrations. As summarised in Table3, the percent bioaccessibility varied across the different PTEs studied. The percent bioaccessible for the PTEs are; 0.4- 24.5 % for As, 20- 94 % for Cd, 1.1-2.7 % for Cr, 4.3- 33.7 % for Cu, 2.8- 24.8 % for Mn, 0.0- 17.7 % for Ni, 8.0- 41.3 % for Pb and 16.4- 93 % for Zn. It is shown from theresults that Cd and Zn had the highest percent bioaccessibility which implies they were the most solubilised in the human body. On the otherhand, Pb and Mn were observed to be moderately bioaccessible while As, Cr and Ni were the PTEs with the lowest percent bioaccessibility which means that Cr and Ni were not easily solubilised in the gastrointestinal environment of the body, hence could not be absorbed into the blood stream and get into other organs of the body to cause any health hazard but rather retained in the human body and ejected eventually. Similar report [20] following the same SBET protocol in their study observed the bioaccessibility rates of As-contaminated soils along railway corridor in Australia to be low.

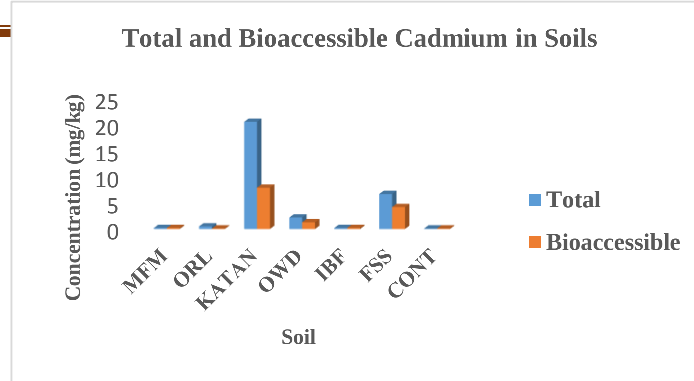
It was also observed that although the percent bioaccesibility of some PTEs (e.g. As and Pb) were moderately low as seen in Table 3, their bioaccessible concentrations were found to be very high when compared to USEPAtolerable oral ingestion rate. A similar result was reported [21] for As (11.3%) and Pb (39.1 %) in a study on soils from Guangzhou City, China. Further, Li et al. [22] in their study of the bioaccessibility and the human health risks of Sb and As in soils from Xikuangshan (XKS) Sb mine, Hunan, China also reported very low percent less than 30% for As and Sb.This is an indication that when using oral bioaccessibility studies for evaluation of risk, the level of potential health risk of the PTEs cannot be determined only by the percent bioaccessibility but rather the bioaccessible concentration of the PTEs in the soils.

Table 3: Percent Bioaccessibility of PTEs in soils

Soil	PTEs	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Total	1.1	0.2	24.2	20.3	131	8.3	36.9	172
	Bioaccessible	0.1	0.2	0.3	4.5	32.4	0.0	12.4	101
	%Bioaccessible	**13.1**	**94.8**	**1.4**	**22.0**	**24.6**	**0.0**	**33.5**	**58.8**
ORL	Total	16.7	0.5	2430	583	3050	1060	165	2790
	Bioaccessible	0.1	0.1	2.30	25.0	193	3.3	13.3	456
	%Bioaccessible	**0.4**	**20.4**	**0.1**	**4.3**	**6.3**	**0.3**	**8.0**	**16.4**
KATANG	Total	19.7	20.5	193	15300	951	140	6380	4900
	Bioaccessible	1.9	7.9	4.40	2990	185	24.9	874	1960
	%Bioaccessible	**9.7**	**38.3**	**2.3**	**19.5**	**19.5**	**17.7**	**13.7**	**40.0**
OWD	Total	18.9	2.2	1370	4120	2340	268	235	2630
	Bioaccessible	0.2	1.3	1.60	328	65.0	2.7	57.0	1120
	%Bioaccessible	**1.1**	**58.1**	**0.1**	**8.0**	**2.8**	**1.0**	**24.2**	**42.5**
IBF	Total	1.0	0.2	29.7	58.1	296	9.8	59.9	428
	Bioaccessible	0.1	0.2	0.40	19.6	64.3	0.0	23.9	270
	%Bioaccessible	**11.0**	**86.0**	**1.4**	**33.7**	**21.7**	**0.0**	**40.0**	**62.8**
	Total	4.3	6.7	120	538	636	84.9	448	2710

FSS	Bioaccessible	1.1	4.2	3.20	95.0	130	2.8	185	1320
	%Bioaccessible	**24.5**	**62.9**	**2.7**	**17.6**	**20.5**	**3.3**	**41.3**	**48.8**
CONT	Total	2.3	0.0	25.0	14.5	232	6.8	17.1	71.7
	Bioaccessible	0.1	0.0	0.1	2.8	55.0	0.0	3.30	68.4
	%Bioaccessible	**4.7**	**0.0**	**0.3**	**19.2**	**23.7**	**0.0**	**19.3**	**93.3**
USEPA	**TDI_{oral}**	0. 3	N/A	15	160	N/A	12	3.6	600


Total and Bioaccessible Arsenic in Soils
Concentration (mg/kg)
20
15
10
5
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Cadmium in Soils
Concentration (mg/kg)
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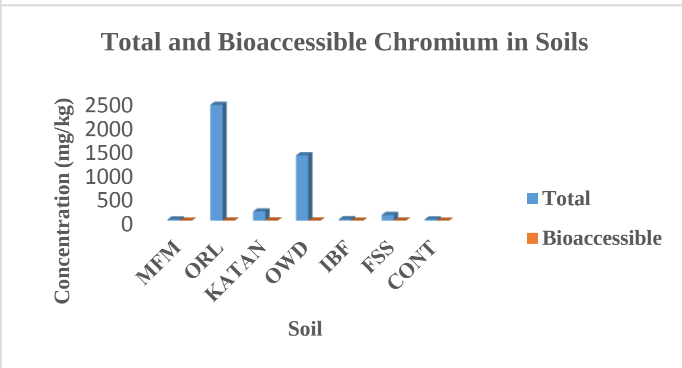
20
15
10
5
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Chromium in Soils
Concentration (mg/kg)
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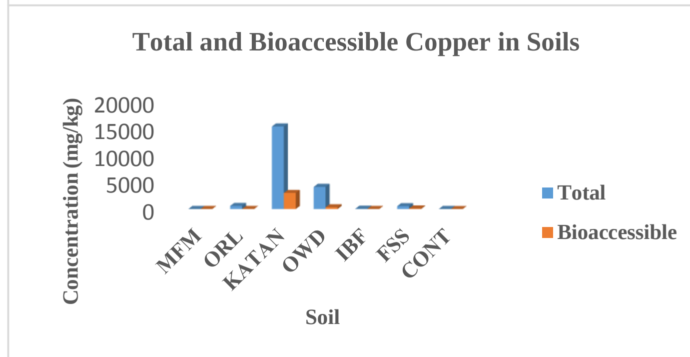
2000
1500
1000
500
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Copper in Soils
Concentration (mg/kg)
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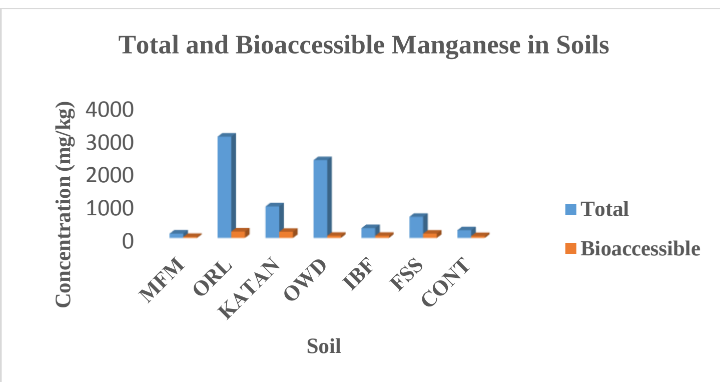
15000
10000
5000
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Manganese in Soils
Concentration (mg/kg)
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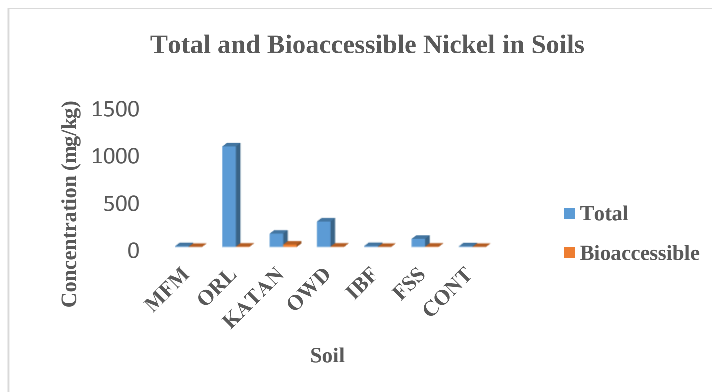
3000
2000
1000
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Nickel in Soils
Concentration (mg/kg)
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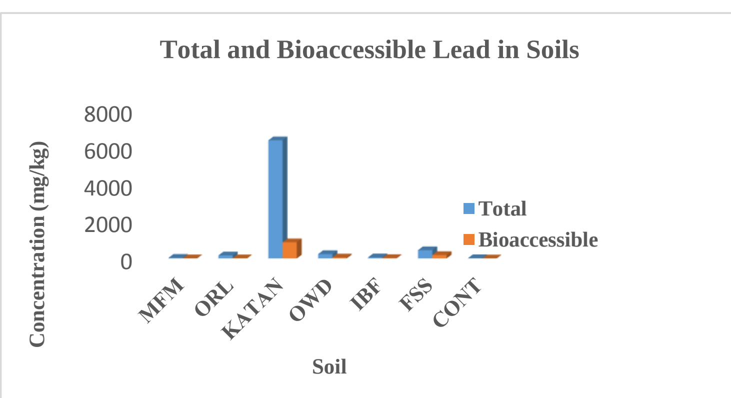
1000
500
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible


Total and Bioaccessible Lead in Soils
Concentration (mg/kg)
8000
6000
4000
2000
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible

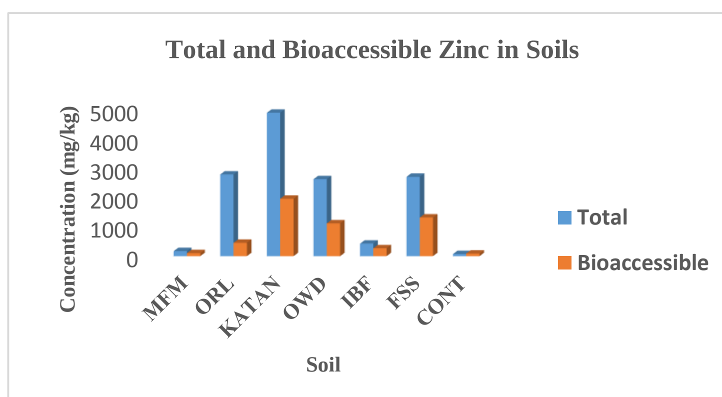

Total and Bioaccessible Zinc in Soils
Concentration (mg/kg)
5000
4000
3000
2000
1000
0
MFM
ORL
KATAN
OWD
IBF
FSS
CONT
Soil
Total
Bioaccessible

Fig 1: Comparison of Total and Percent Bioacessibility of PTEs in Soils

Statistical Analysis of Data (Pearson Correlation Analysis)

Table 4: Pearson Correlation Coefficient (r) for individual PTEs between total and respective bioaccessible concentrations

r	***Total As***	***Total Cd***	***Total Cr***	***Total Cu***	***Total Mn***	***Total Ni***	***Total Pb***	***Total Zn***
SBET As	0.4028	0.97227*	-0.2809	0.8268*	-0.1186	-0.1778	0.8848*	0.7945*
SBET Cd	0.4622	0.9801*	-0.2506	0.8610*	-0.0749	-0.1762	0.8954*	0.8224*
SBET Cr	0.6667	0.8556*	0.1804	0.7351*	0.3312	0.2653	0.7515*	0.9613*
SBET Cu	0.5867	0.9553*	-0.1559	0.9871*	-0.0036	-0.0943	0.9956*	0.7589*
SBET Mn	0.6520	0.6039	0.4842	0.5184	0.5418	0.6481	0.5738*	0.8283*
SBET Ni	0.6339	0.9651*	-0.0653	0.9748*	0.0818	0.0176	0.9924*	0.8242*
SBET Pb	0.5164	0.9920*	-0.2211	0.9542*	-0.0654	-0.1313	0.9899*	0.7846*
SBET Zn	0.6618	0.8870*	0.0051	0.8178*	0.2102	-0.0112	0.7743*	0.9195*

*values Significant at 95% confidence level ($p < 0.05$)

The results of the correlation analysis between measured total PTE concentration and their respective SBET bioaccessible concentration are as presented in Table 4. Strong positive correlations ($p < 0.05$) were observed for Cd, Cu, Pb and Zn. However, Cr, Ni and Mn showed limited correlation between the total and respective SBET bioaccessible concentrations.A cross-element correlation analysis showed strong positive correlation ($r > 0.8$) between Cd and As, Cr, Cu, Ni, Pb, Zn. Similarly strong correlation was observed between Cu and As, Cd, Ni, Pb, Zn. Further, strong correlation exists between Pb and As, Cd, Cu, Ni, Pb. Also, Zn had strong correlation with the other PTEs.Negative correlation was observed between Cr, Ni, Mn and the other PTEs (As, Cd, Cu, Pb).

CONCLUSION

This study revealed that though the PTE concentrations in soils investigated were very high, their bioaccessible fractions were relatively low.Amongst the PTEs, Cd and Zn showed higher percent bioaccessibility (up to 90%), Pb and Mn had moderate bioaccessibility level whilst As, Cr and Ni had the lowest percent bioaccessibility (< 20%). Comparison of bioaccessible PTEs levels with toxicological data indicated that Cd, Pb and Zn were PTEs of greatest concern. This could be linked to the anthropogenic activities at the sites.The experiment has demonstrated the importance of considering the bioaccessible data rather than the total PTEs data for health risk calculations, because it could provide more realistic evaluation ofhuman health risks. It is recommended that proper monitoring and isolation of dumpsites from residential areas should be done by the government and local agencies in order to forestall either accidental ingestion of soils by adults or direct ingestion by children through crawling on soils and more importantly their hand to mouth behaviour.

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