J. Chem. Soc. Nigeria, Vol. 49, No. 1, pp 066 - 078 [2024]

SOLID-PHASE FRACTIONATION OF HEAVY METAL IONS IN SOILS FROM MUNICIPAL WASTE DUMPSITE IN PORT HARCOURT, NIGERIA

C. C. Obunwo, S. C. Ubah*, O. S. Bull and P. M. Amaibi
Department of Chemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria
*Correspondence: ubahchinonyelum96@gmail.com, +2347061913760

ABSTRACT

In many cities across the developing countries solid wastes are disposed indiscriminately without recourse to the human health risk. Heavy metals found insolid wastes may likely be released into the environment via flood runoff. To assess whether the contaminants can affect the groundwater, soil samples were collected for analysis in two seasons – dry and wet seasons. To determine level of the metals in the soil matrix, the samples were subjected to acid digestion followed by atomic absorption spectroscopy analysis. The different solid-phases associated with heavy metals were evaluated from the soil samples by using sequential extraction method. The geo-accumulation index and mobility factor were considered to assess the level of contamination and migration of the metals present in the soil. The soil metal contents strongly correlated with the soil pH and organic matter. The exchangeable fraction has Cr > Pb > Co in the orderof its mobility in the soil, whilst in the residual fraction suggesting the most immobile species with Pb > Co > Cr > Ni present in the soil. The geo-accumulation index showed that the degree of pollution of the dumpsite during the dry and wet seasons was mostly unpolluted. The study indicates that the metals of interest may not likely pose environmental risk due to their relatively low concentrations and the chemical species in which they exist in soil.

Keywords: Soil, Dumpsite, Heavy metals, Solid-waste, Sequential extraction, Speciation

INTRODUCTION

In most urban environment waste management is a major concern since the indiscriminate disposal of waste can result to disease outbreak. Municipal solid wastes are derived from domestic and commercial activities within urban areas. Many cities in the developing countries dispose of their wastes in designated areas and are further removed for incineration ordeposited into a landfill.

Contaminants found in solid wastes can be released into the environment via flood runoff which may pose severe threat tohuman health [1,2]. These contaminants include heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), nickel and lead (Pb). Heavy metals are non-degradable, persistent, and toxic elements in nature and have been known tocause a wide range of neurological and nervous diseases [3,4]. The levels of the heavy metals in soil have been attributedto human activities associated with urbanization and industrialization. Traditionally, previous studies have been reported on elevated level of metals in soils, but these findings may not provide better understanding of the mobility of the elements [5-7].

However, the fate and behaviour of metals in soil have been studied on the basis of chemical speciation. The behaviour of the metals critically depends on the form in which they occur in soil. The chemical form in which the element is bound to the solid matrix influences its mobility, bioavailability and toxicity to organism [8].

The metal ions in soil can be fractionated into different solid phases i.e. exchangeable, carbonate, organic, iron and manganese oxides as well as sulphide minerals. However, the different solid- phases can be released from the soil matrix using sequential extraction methods. Many extraction methods to study chemical speciation have been developed by different researchers [9-11]. The BCR method comprises three extraction (exchangeable, reducible and oxidizable) phases but the most commonly method proposed by Tessier, Campbell and Bisson in 1979 has five sequential extraction phases. The fractional phases in the Tessier method include exchangeable, carbonate, organic, iron and manganese oxides and residual fraction. This method has been used to study several environmental matrices (soil, sediment and fly ash) but not necessarily determining the status of the pollution. Accumulation of heavy metals in soil when elevated above certain guideline values can lead to contamination.

To classify the degree of pollution of heavy metals in soils, the geo-accumulation index uses the total concentration of the metal found within the area.

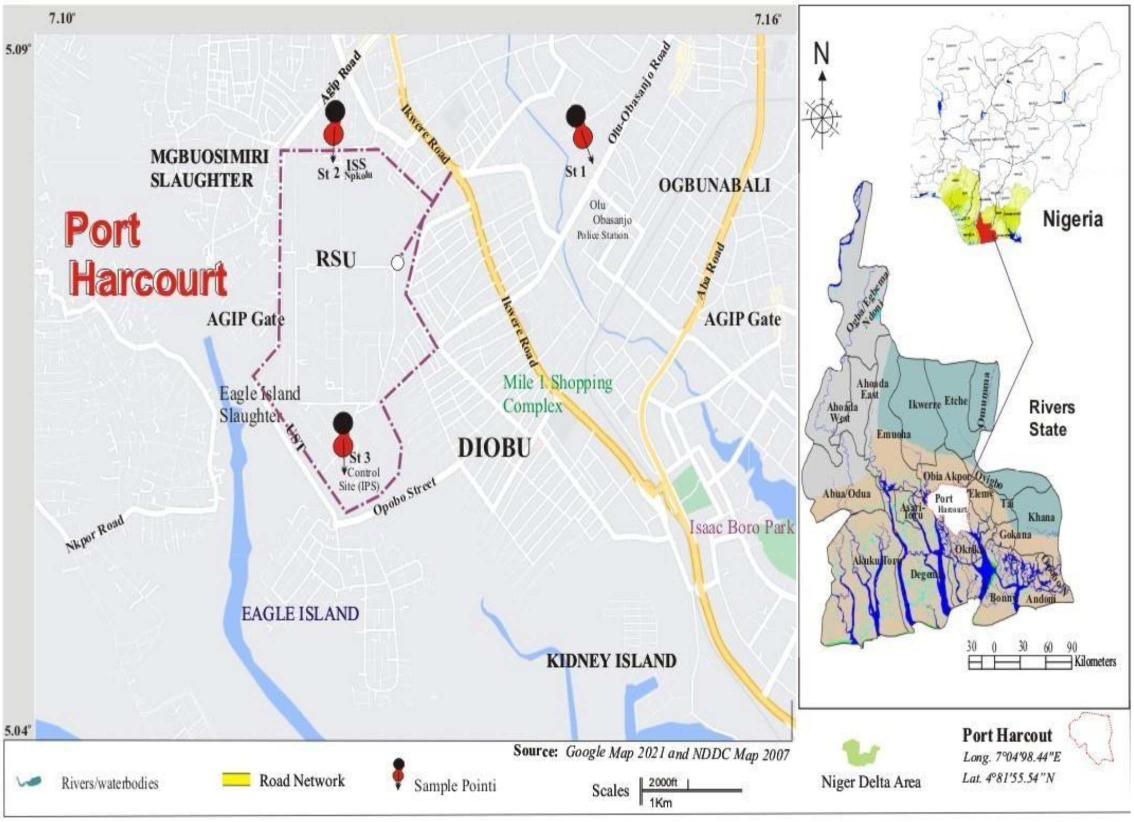
In this study, we measure the physicochemical parameters of the soil as well as total soil contents of some metals. We also employ the Tesser's sequential extraction method to evaluate five fractionating phases of the metals. The influence of the seasonal variation and soil profile on mobility of metals has also been considered in this study. The findings in this study may deepen our understanding of the behaviour of metals in soil matrix.

MATERIALS AND METHODS

Study Area

Port Harcourt city is an industrial urban area located in the Southern part of Nigeria, with a dense population, approximately 3 million people. Majority of the people living in this city are involved in commercial activities, which results to huge solid waste deposit especially within the Nkpolu-Oroworukwo(Mile 3) Diobu area as presented in Figure

1. This area is situated between latitude 270° West, NL:- 4°48'14" and longitude EL:- 6°59'49" having a dumpsite along a major road in the city. The sampling site is found within highly busy commercial area at Olu Obasanjo Road in Port Harcourt. Several mechanic workshops are situated in this area with a few residential buildings as well as a cassava plantation.



Source: *Google Map 2021 and NDDC Map 2007*

Figure 1: Location of Study Area

The soil is mainly sandy with greasy oil contents linked to the common activities operating in the area. Within the vicinity ofthe study area, a small water body from an interurban Creek is about 5 m away from the sampling site.

The climate of this area is classified to be a tropical rain forest region of which twoclimatic seasons are typically observed peryear, the dry season (December to March) and wet season (April to November). Due to high rain fall some areas experience flash flooding that can cause run-off and gradually distribute potential toxic element to other parts away from the point source.

Soil sampling and sample pre-treatment

Soil samples were collected in two seasons(dry and wet) from soil profile levels using hand auger. At the top level ranging from 0-15 cm, soil samples were taken and transferred in a polythene bag during the dry and wet seasons. A sub-soil sample from 15 -30 cm depth was collected in a polythene bag during the two seasons under this investigation. For purposes of quality control, samples were collected from an area known to be a non-dumpsite.

The total of eight (8) samples taken to laboratory were subjected to air drying at ambient temperature for four (4) days priorto grinding and then sieved into small particle size < 2 mm using nylon mesh. Analiquot of the sample was taken for further chemical analyses.

Sample preparation and characterization

A portion of the sample was accurately weighed to measure the soilphysicochemical parameters including pH, electrical conductivity, organic matter, total exchangeable acidity and soil texture. The procedures to conduct each of the parameters were previously described elsewhere [12]. Each sample was analysed to determine soil pH by measuring soil: water ratio of 1:2.5 using a pH meter. Soil electrical conductivity (EC) was measured on soil: water suspension in which 10 g sample was added in a 25 ml distilled water, thoroughly homogenised in beaker and the reading taken using an electronic probe. A standard method (Walker and Black method) was adopted to determine the total organic carbon (TOC). The detailed procedure can be found in a study conducted on soil [13]. Freshly prepared $K_2Cr_3O_7$ (5 ml) was added to 0.5 g soil in a conical flask. Following the addition of 10 ml H_2SO_4, the solution was agitated and 5 ml $K_2Cr_3O_7$ added to the mixture. The solution was allowed to settle for 15 minutes followed by addition of 250 ml distilled water and 12.5 ml $FeSO_4$ solution to the soil mixture. The mixture was then titrated against standard potassium permanganate. The values obtained were multiplied by 1.724 to give the total organic carbon.

Following a titration of a soil mixture against 0.05 N HCl as described in a procedure detailed elsewhere [14] the total exchangeable acidity was calculated for each analysed sample.

Soil particle size analysis was conducted to distribute the soil into sand, silt and clay texture. The ASTM D4318 hydrometer standard method [15] was employed forparticle size analysis of the soil. Heavy metal determination was performed by acid digestion method followed by using atomic absorption spectroscopy analysis. A 15 ml solution of HCl and HNO_3 in ratio 3:1 v/v, respectively was added to 0.5 g soil sample and digested ina hot plate for 45 minutes. The mixturewas allowed to cool and filtered in 50 ml volumetric flask using a whatman filter paper. The solution was made up to mark with distilled water. The analysis of the concentration of heavy metals was carried out on each sample solution using AAS.

Fractionation of heavy metals insoils

A chemical fractionation of the heavy metals present in the soil was determined by the Tessier method [16]. The summary procedure as described previously is presented in Table 1.

Geo-accumulation index (Igeo)

The soil metal content was calculated to identify the level of environmental concern and represented as geo-accumulation index (Igeo). The index was originally developed by Muller [17] toevaluate metal contamination in soil and dust.

The Igeo index as expressed in equation 3 is shown below:

$$\text{Igo} = \log 2\left(\frac{\text{Cn}}{1.5\text{Bn}}\right) \qquad (3)$$

Where: C_n = measures the concentration of the metal in soil

B_n = the background geochemical value of the metal

Table 1: Summary of sequential extraction procedure

Fraction/Step	Extraction reagents	Time/Temperature	Soil: solution ratio
Water/exchangeable (F1)	M NH4NO3 (pH = 7)	1 h, 25ºC	1:25
Carbonate (F2)	1.0 M NaOAC in 25% HOAC (pH = 5)	6 h, 25ºC	1:25
Fe/Mn oxide (F3)	0.04 M NH4OH HCl in 25% HOAC (pH =2)	3 h, 95ºC	1:25
Organic matter (F4)	1. 0.01M HNO3/30% H2O2 (pH = 2)	2 h, 85ºC	1:25
	2. 30% H2O2 (pH = 2)	3 h, 95ºC	
	3. 3.2 mol/L NH4OAC in 20% HNO3	0.5 h, 25ºC	
Residual (F5)	HF – HNO3 – HClO4 (v/v/v + 1:3:2)	180ºC near dryness	1:50

The constant 1.5 is considered as natural fluctuation for given substance in the environment to be detected by small anthropogenic inputs. The categories of theIgeo defined by Muller have seven classes as described in Table 2.

Table 2: Class of geo-accumulation indexes to identify pollution level

Class	Value	Status
0	Igeo < 0	Unpolluted
1	0 < I geo < 1	Unpolluted to moderately polluted
2	1 < I geo < 2	Moderately polluted
3	2 < I geo < 3	Moderately to strongly polluted
4	3 < I geo < 4	Strongly polluted
5	4 < I geo < 5	Strongly polluted to extremely polluted
6	I geo > 5	Extremely polluted

The total concentration of each metal of interest was adopted to calculate the Igeo index within the study area.

Mobility factor

To evaluate the mobility factor (MF) of the heavy metals, the fractions of the soil content of the metal obtained from the sequential extraction procedure was employed, excluding that of the residual fraction. Each fraction is assigned a weightas described in the equation 3 below:

$$\mathrm{MF} = \frac{\mathrm{F1} + \mathrm{F2}}{\mathrm{F1} + \mathrm{F2} + \mathrm{F3} + \mathrm{F4} + \mathrm{F5}} \times 100$$

(4)

Statistical Analysis

A statistical analysis was performed on all datasets to compute the mean values,standard deviation and Pearson correlation matrix for the parameters of interest. The analysis was conducted using Microsoft excel Window 2013 and Minitab statistical package version 18. To the soil quality within the study area, geo-accumulation indexes and element mobility ratio were calculated by use of formulae as reported in previous studies [18].

RESULTS AND DISCUSSION

Soil properties and heavy metals concentrations

Soil samples from the dumpsite weresubjected to chemical analyses for physicochemical parameters and the results are presented in Table 1.

The soil pH was statistically significant ($p<0.05$) from dry to wet seasons along thesoil profile (Table 1). Soil pH ranged between 5.74 and 6.19, indicating slightly acidity

While the results from other soil propertiessuch as electrical conductivity (EC), organic matter and total exchangeable acidity were not statistically significant. The soil particle size was more of sandfraction (Table 3).

Concentration of heavy metals varied fromeach of the seasons and soil profile as shown in Table 3.

The results indicated that the lowest concentrations of heavy metals in the dry season along the topsoil were Ni and Co soil contents (0.94 mg/kg and 0.09 mg/kg, respectively).

Highest concentration of Cr (11.38 mg/kg) was found in the dry season within thebottom soil. Of all the study elements, soil Cr content was lowest during the wet season.

The unexpected outcome may be attributed to formation of recalcitrant complexes.

This result is consistent with that ofprevious study which showed that Cr concentration declines as the soil acidity increases [19].

Soil profile/season	**pH**	**EC (µS/cm)**	**SOM (%)**	**TEA (Mol/kg)**	**Soil texture (%)**			**Ni**	**Pb**	**Cr**	**Co**
					Sand	**Silt**	**Clay**				
Top-dry	5.74 ± 0.61	2640 ± 23.09	3.77 ± 0.51	1.33 ± 0.001	91.4±8.210	7.0±1.010	1.6 ±0.001	0.94 ± 0.02	2.19 ± 0.01	9.76 ± 0.01	0.09 ± 0.01
Bottom-dry	6.03 ± 1.35	280 ± 5.50	2.15 ± 0.10	1.52 ± 0.001	95.8 ±5.012	2.6 ±0.003	1.6±0.001	1.12 ± 0.02	2.94 ± 0.01	11.38 ± 0.01	0.39 0.01
Top-wet	6.08 ± 1.52	3250 ± 1.52	5.85 ± 0.42	1.36 ± 0.001	98.4±6.02	1.0 ±0.001	1.6±0.001	1.86 ± 0.01	3.60 ± 0.01	4.56 ± 0.01	0.63 ± 0.01
Bottom-wet	6.19 ± 1.1.73	2850 ± 1.52	3.97 ±0.10	1.44 ± 0.010	84.4±5.21	7.8 ±1.010	6.8±0.501	1.14 ±0.01	3.98 ±0.01	4.50 ± 0.01	2.02 ±0.01
p-value	0.002	0.085	0.461	0.054							

Soil properties and heavy metal concentrations

Pearson Correlation Matrix between soil properties and heavy metal concentrations

	pH	*EC*	*SOM*	*TEA*	*Sand*	*Silt*	*Clay*	*Ni*	*Pb*	*Cr*	*Co*
pH	1										
EC	0.043118	1									
SOM	0.171016	**0.884444**	1								
TEA	0.522494	**-0.81507**	**-0.71611**	1							
Sand	-0.19333	-0.23911	0.190677	-0.05545	1						
Silt	-0.19065	0.239719	-0.23956	-0.16017	**-0.92613**	1					
Clay	0.624977	0.295852	0.015406	0.214698	**-0.88174**	0.643614	1				
Ni	0.443898	0.414976	**0.783235**	-0.22506	0.589065	**-0.74966**	-0.20488	1			
Pb	0.9626	0.312056	0.398664	0.277199	-0.25651	-0.10848	0.680535	0.52796	1		
Cr	-0.62527	**-0.80573**	**-0.80816**	0.332683	0.264854	-0.03831	-0.57286	-0.6168	**-0.81266**	1	
Co	**0.795189**	0.318213	0.14589	0.260411	**-0.73014**	0.427663	**0.96597**	0.035072	**0.846826**	-0.69847	1

A summary of the average concentration of heavy metals from dry and wet seasons is presented in Figure 1.

The pH is considered as one of the most important factors that influences the mobility and distribution of metals in soil. The distribution of soluble or insoluble phases of Cr may have been affected by formation of organic Cr complexes [20,21], adsorption to particle and biological activities (e.g. suphate reducing bacteria) in the dumpsite. Further study on solid phase fractionation is significant in understanding the mobility and distribution of the metal speciation.

Fractionation of heavy metals

A 5-step sequential extraction procedure was adopted to separate metals phases into exchangeable, Fe-Mn oxide, carbonate,organic and residual. The average concentrations of metals from the soil samples are displayed in Figure 1.

The study highlights the distribution of the elements into various fractions Sequential extraction procedure was adopted to fractionate trace elements present in a soil matrix. This method is widely used to understand the different solubility of the elements related to mineral phases. The predominant fraction of soil Ni contents were in the carbonate phase (Figure 2).

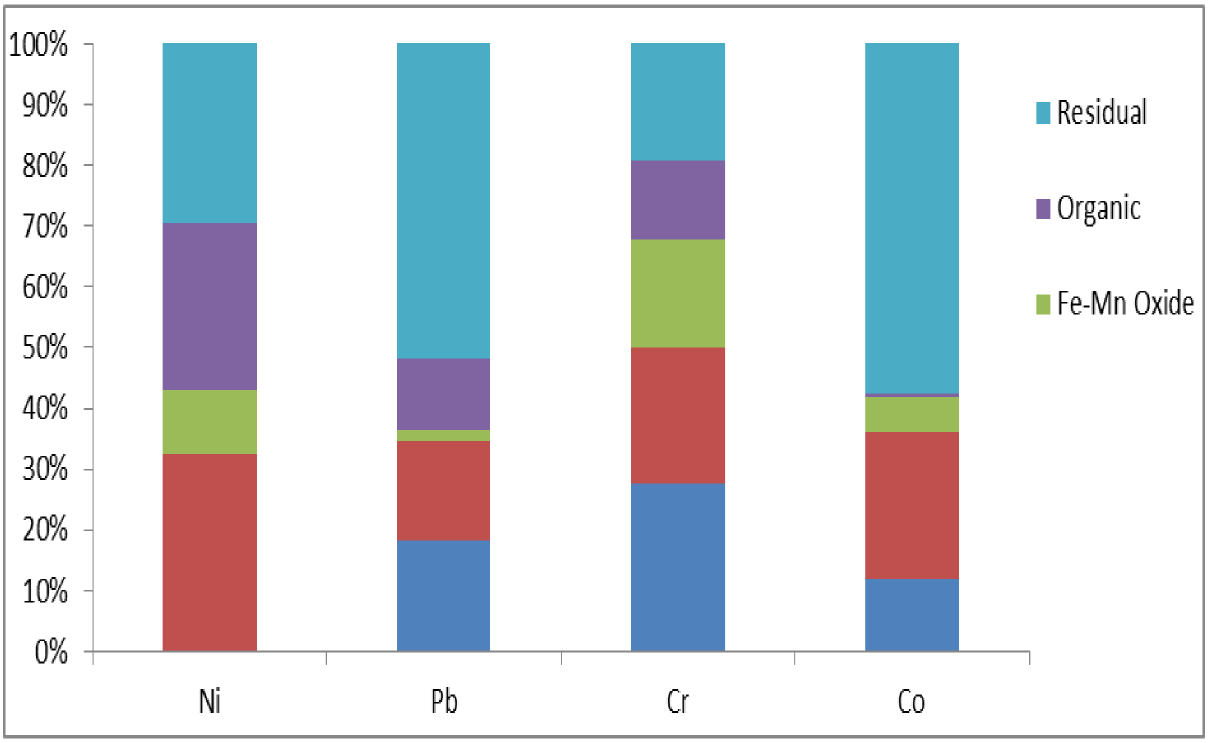


Figure 2: Soli-phase distribution of heavy metals fractions in soil sample

This fraction is commonly bound by covalent forces, and it is difficult to displace the metal from the solid phase.The metal is precipitated or co-precipitated by carbonate to serve as an adsorbent where the organic and Fe-Mn oxide phases are in less abundant [22].

The carbonate phase is susceptible to pH changes in which state of the dumpsite can influence the release of Ni content in greater proportion.

The amount of Pb associated with residual fraction is similar to that of previous studies [23]. This fraction is mainly bound by silicate and other crystalline minerals that are insoluble in strong acids. The result suggests that Pb is immobile in the soil and may likely be derived from the parent materials.

Cr appears to be primarily associated with the exchangeable phase. In this Cr may be of concern since highest proportion wasfound in most mobile phase. The exchange phase indicates that the metal is weaklyadsorbed and retained on the solid surface by electrostatic interaction and the metal can be easily released by ion exchangeable processes. Therefore, the result suggests that Cr is mobile and bioavailable for plantuptake.

The soil Co content indicated a high presence of residual phase in the soil. The residual may not be bioavailable and mobile; thus, it may be less harmful to the environment. However, the presence of Cr may also be attributed to anthropogenic inputs considering the nature of the study site. Geo-accumulation index (Igeo)

Concentrations of studied elements werehigher in the wet season than in the dry season except for Cr. The results from the wet season at topsoil level were consideredto compute geo-accumulation index for each of the elements as presented in Table 3.

Cobalt soil content appeared moderately polluted from the topsoil while the other elements including Cr was considered unpolluted at the site. Residual fraction of Co was highest in this study to account for the moderately polluted index observed especially during the wet season. Fractionation of the elements has shown that Pb and Co contents were more in the residual fraction (Figure 2). The residual fraction is less likely mobile but can accumulate on the topsoil over a long period of time. These results corroborate the findings from previous study on heavy metals in soils by Izah et al. [24] The accumulation of metals on the topsoil can be attributed to human activities. The variations in the Igeo data account for penetration capacity of these elementsacross the soil profile with respect to the chemical speciation and soilcharacteristics. From the study, the accumulation of heavy metals in the topsoil may suggest unpolluted to moderately polluted condition for Cr and Co present in the soil.

Mobility factor of heavy metals in soil

The mobility factor of the metals in the soil was determined by a method described in previous studies. The mobility factor of heavy metals is considered to account for the possible contamination of thegroundwater. The heavy m metals found with the highest mobility include Ni, Pb, Cr and Co as presented in Figure 3.

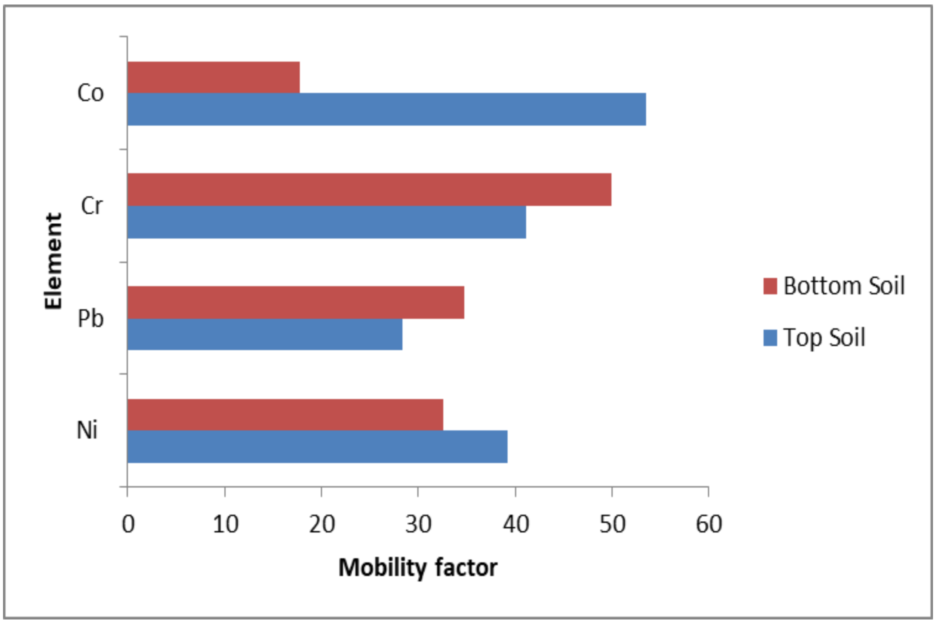


Figure 3: Element mobility between soil profiles

The results from the investigation showed that metals migrate more during the dry season which may likely be attributed to activities of microorganisms in the soil. Amongst the studied elements Co has the highest mobility followed by Cr and Nipresent in the topsoil especially during the dry season. It is important to note that since the topsoil indicated more migration of the heavy metals, the groundwater may be safe from contamination. Heavy metals present in soil may be found to migrate from the point source to other areas due to the relative high mobility factor.

As expected, the wet season mobility factor appeared to be low compared to the dry season. The unexpected result may be accounted for by high microbial activities in active dumpsites. In this study, the presence of Pb and Ni has shown relativelylow mobility (Figure 2). This result suggests that immobile fractions are more likely to stabilise these metals in the soil. High mobility of Cr and Co found in this study is like those reported previously for sludge samples [25].

The total concentration of Heavy metals in soil may not only be sufficient to determine the toxicity and bioavailability but chemical speciation of metal is an important factor to consider in terms of the mobility and bioavailability of metals. The chemical forms in which metals exist in soil described by the various fractions can deepen our understanding of how the elements migrate across the soil profile.

CONCLUSION

Despite the total concentration of heavymetals and the soil properties, there arenumber of factors influencing the mobility and availability of these elements in soil. Dumpsites are commonly rich in organic matter, as it is expected, the soil metal contents strongly correlated with the soil pH and organic matter. At low soil pH the soil releases more metals from its complexes, hence resulting to high mobility. However, the mobility of the metal may not only depend on the soil properties but on the chemical speciationof the element.

To highlight the distribution of the studied elements into chemical fractions, the exchangeable fraction has Cr > Pb > Co in the order of its mobility in the soil. While, the residual fraction as described to be the most immobile fraction decreases in order of Pb > Co > Cr > Ni. From the study, the accumulation of heavy metals in the topsoil may suggest unpolluted to moderately polluted condition for Cr and Co present in the soil. The chemical forms in which metals exist in soil described by the various fractions can deepen our understanding of how the elements migrate across the soil profile.

Although the total concentration of heavy metals suggests level of the contaminants in soil, it may not determine if the contaminants can affect the groundwatersince the fate and behaviour of the elements are influenced by a number of factors. The migration of metals at the level of the topsoil may likely not contaminate the groundwater nor pose environmental risk. Further study onthe surface water within this vicinity may be investigated to assess the impact of the runoff on the environment.

REFERENCES

1. E. Amasuomo, and J. Baird (2017). Solid waste management trends in Nigeria. *British Journal of Environmental Sciences. 6*, 25-37.
2. M. Asase, E. K. Yanful, M. Mensah, J. Stanford, and S. Amponsah (2009). Comparison of municipal SWMS in Canada and Ghana, A case study of the cities of London, Ontario, and Kumasi, Ghana. *Waste Management.29(10)*, 2779-2786.
3. A. Asati, M. Pichhode, and K. Nikhil (2016). Effect of heavy metals on plants, an overview. *International Journal of Application or Innovation in Engineering and Management.5(3*), 56-66.
4. A. H. Baghaie, and M. Fereydoni (2019). The potential risk of heavymetals on human health due to the daily consumption of vegetables. *Environmental Health Engineering and Management Journal. 6(1)*, 11-16.
5. J. Korzeniowska, E. Stanislawska- Glubiak, and J. Igras (2011). Applicability of energy crops for metal phytostabilization of soils moderately contaminated with copper, nickel and zinc. *Journal of Food Agriculture andEnvironment. 93(4)*, 693-697.
6. C. J. Ugboma, T. Sampson, and N. E. Mbonu (2020). Bioremediation of heavy metals from artisanal crude oil refinery (Kpo fire) impacted soil using *Bacillus flexusand Pseudomonas aeruginosa* inNgie Community, Degema Local Government Area, Rivers State, Nigeria. *Journal of Applied Science and Environmental Management*. 24(12*)*, 2049-2054.
7. D. B. Onwuna, H.O. Stanley, G.O. Abu, and O. M. Immanuel (2022).Impact of artisanal crude oil refinery on physicochemical and microbiological properties of soil and water in Igia-Ama, Tombia Kingdom, Rivers State, Nigeria. *Asian Journal of Environment and Ecology*. *19(3*), 56-67. DOI:10.9734/ajee/2022/v19i3412

8. J. R. Bacon, and C. M. Davidson (2008). Is There a Future for Sequential Chemical Extraction? Analyst. *133*, 25-46. https,//doi.org/10.1039/B711896A

9. D. M. Templeton, and H. Fujishiro (2017). Terminology of elemental speciation – an IUPAC perspective. Coord. Chem. Rev. 352, 424–431.doi, 10.1016/j.ccr.2017.02.002

10. Galceran, J. Y. Gao, J. Puy, M. Leermakers, C. Rey-Castro, C. Zhou and W. Baeyens (2021). Speciation of Inorganic Compounds in Aquatic Systems Using Diffusive Gradients in Thin-Films, A Review Front.Chem. *9*, https,//doi.org/10.3389/fchem.2021.624511

11. O. Giuffrè, A. Napoli, and C. Rey-Castro (2021). Advances in analytical techniques and methodology for chemical speciation study. Front. Chem. *9*, https,//doi.org/10.3389/fchem.2021.692144.

12. P. M. Amaibi, J. A. Entwistle, N. Kennedy, M. Cave, S. J. Kemp, S. Potgieter-Vermaak and J. R. Dean. (2019). Mineralogy, solid-phase fractionation and chemical extraction to assess the mobility and availability of arsenic in an urban environmental. *Journal of Applied Geochemistry*. 100, 244-257.

13. P. Jha, A. K. Biswas, B. L.Lakaria, R. Saha, M. Singh, and A. S. Rao (2014). Predicting Total Organic Carbon Content of Soils from Walkley and Black Analysis. Communications in Soil Scienceand Plant Analysis. *45*,713–725.

14. K. A. B. Logan, M. J. S. Floate and A.D. Ironside (2008). Determination of exchangeable acidity and exchangeable aluminium in hill soils part 1 exchangeable acidity. Communications in Soil Scienceand Plant Analysis. *16*, 301-308.

15. ASTM (2022). ASTM D7928-21e1: Standard test method for particle-size distribution(gradation) of fine-grained soilsusing the sedimentation(hydrometer) analysis. https//www.astm.org/d7928-21e01.html

16. A. Tessier, P. G. C. Campbell, M. Bisson (1979). Sequential extraction procedure for the speciation of particulate trace metals. Analytical chemistry - ACS Publications.

17. G. Muller (1979). Index of Geo-accumulation in Sediments of the Rhine River. *Journal ofGeology*. 2(3), 108-118.

18. H. M. Zakir and N. Shikazono (2011). Environmental mobility and geochemical partitioning of Fe, Mn, Co, Ni and Mo in sediments of an urban river. *Journal of Environmental chemistry and Ecotoxicology. 3(5),* 116-126,

19. P. Du, Y. Xie, SS. Wang, H. Zhao, Z. Zhang, B. Wu, F. Li (2015). Potential sources of and ecological risks from heavy metalsin agricultural soils, Daye City, China. Environmental Science and Pollution Research. *22*, 3498- 3507.

20. M. Assfalg, I. Bertini, M. Bruschi, C. Michel, P. Turano (2002). The metal reductase activity of some multiheme cytochromes c, NMR structural characterization of the reduction of chromium(VI) to chromium (III) by cytochrome c7. Proceedings of the National Academy of Sciences. *99 (15)*, 9750-9754.

21. G. J. Puzon, A. G. Roberts, D. M. Kramer, L. Xun (2005). Formation of soluble organo− chromium (III) complexes after chromate reduction in the presence of cellular organics. *Environmental Science and Technology. 39 (8)*, 2811-2817.

22. C. N. R. Rao, A. K. Cheetham and A. Thirumurugan (2008). Hybrid inorganic–organic materials, a new family in condensed matter physics. Journal of Physics, Condensed Matter. *20 (8)*, 083202,

23. Q. M. Jaradat, A. M. Massadeh, M. A. Zaitoun and B. M. Maitah.

(2006). Fractionation and sequential extraction of heavy metals in the soil of scrapyard of discarded vehicles. *112*, 197–210.

24. S. C. Izah, S. E. Bassey, E. I. Ohimain (2017). Geo-accumulation index, enrichmentfactor and quantification of contamination of heavy metals in soil receiving cassava mill effluents in a rural community in the Niger Delta. Molecular Soil Biology. 8, 7-20. https://doi.org/10.5376/msb.2017.08.0002

25. A. Janaszek and R. Kowalik. (2023). Analysis of Heavy metal contaminants and mobility insewage sludge-soil mixtures for sustainable agricultural practices. Water. *15(22)*, 3992; https,//doi.org/10.3390/w15223992