



Remediation of Heavy Metals from Petroleum Sludge Impacted Soils using Organic Waste

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ABSTRACT

Soil infectivity caused by heavy metals has increasingly received worldwide attention due to their high toxicity, thus its remediation is a priority. Petroleum sludge impacted soil samples around Warri Refinery and Petrochemical Company (WRPC), Delta State, Nigeria, were subjected to bioremediation of heavy metals (Cr, Cd, Pb, Ni, Cu, Co, Mn, Fe and Zn) using agro-waste from *Moringa Oleifera* seed (3:1) and monitored for contaminants reduction for 90 days at 30 days interval. Results obtained were compared with that of the control soil sample collected 8.5 km away from WRPC. At the end of 90 days, the percentage removal of all the heavy metals was between 65.00 % and 88.32 % which showed a successful bioremediation. Metal indicators like Enrichment Factor (EF) and Geoaccumulation Index (Igeo) were employed to assess the intensity of contamination. At the end of the study, EF revealed no to minor enrichment except for Cr and Pb. The computed Igeo further confirmed that all the studied sites were decontaminated except for Cr and Pb. When the portion of soil to biostimulant was varied (3:2) and observed for another 30 days, the EF and Igeo revealed that addition of higher mix ratio did not produce a corresponding increase in the removal of residual metals as expected. From the results, the applied organic fertilization treatment has proven to be effective to accelerate bioremediation of petroleum sludge impacted soils.

Keywords: heavy metals; toxicity; moringa seed; bioremediation; soil; petroleum

1. INTRODUCTION

Rapid growth of industrialization and extraction of natural resources in developing countries like Nigeria have led to the generation of huge volume of wastes and consequential environmental degradation [1]. In the Niger Delta region of Nigeria being one of the oil-rich states, petroleum industries are renowned for the discharge of large quantities of toxic wastes and untreated effluents to the immediate environment which poses significant impacts to the ecosystem. Poor waste disposal of petroleum sludge, as a direct result of refining activities, affect the properties of soils with the build-up of high concentrations of heavy metals in the affected sites [2,3,4]. Heavy metals contamination in the environment is a major concern due to their toxicity and threat to the environment and human life. As chemical hazards, heavy metals are non-biodegradable and can remain almost indefinitely in the soil environment. However, their availability to biota can change considerably depending on their chemical speciation in the soil [1,5]. Metals are essential to the biological functions of plants and animals but at elevated levels, they interfere with metabolic reactions in systems of organisms. There are well documented health and environmental risks associated with heavy metals, thus its treatment has become a priority [6,7]. Various approaches used to remedy heavy metal contaminated soils include physical and chemical systems like land filling, encapsulation, fixation, leaching, etc, but most of these methods are not economically viable, and they do not ensure restoration without residual effects. Alternatively, bioremediation is a promising field of research with more economically and environmentally sound approach, which gives

reliable and simple technologies over chemical and physical processes. Bioremediation is a technique that utilizes the biological mechanisms inherent in microbes and plants to eradicate hazardous contaminants and restore the ecosystem to its original condition [8,9,10]. It is employed to break down xenobiotics and mitigate toxic heavy metals, by altering them into elements with little or no toxicity, henceforth forming innocuous products [11,12]. With the objective of improving the process of bioremediation, diverse approaches can be employed, dependent of the type of the contaminated environment. One of these approaches, biostimulation, involves encouraging the growth of indigenous microorganisms in the contaminated sites by supplying them with the optimum levels of nutrients essential for their metabolism [13,14,15]. Consequently, the rate of biodegradation can be amplified. The primary advantage of biostimulation is that bioremediation will be undertaken by already present native microorganisms that are well-suited to the environment, and are well distributed spatially within the subsurface [16,17]. Recent advancements have proven successful via the addition of organic fertilizers to increase the bioavailability of microbes within the medium [18,19,20]. The application of organic amendments such as agro-waste from *Moringa Oleifera* seed in metal-contaminated soils could cause differences in the soil microbial population by changing pH, decreasing the solubility of heavy metals and increasing autochthonous microbial biomass. *Moringa Oleifera* seed cake (MOSC) is a byproduct, obtained after oil is extracted from the seeds. These wastes are considered useless to the ordinary man, but research has shown that such wastes are useful material to

modify the soil physical and chemical properties [21]. It is extensively used in various ways: it has been shown to possess effective coagulant properties to flocculate particles in wastewater treatment [22,23], it has biosorption behavior for the removal of toxic heavy metals from water bodies and soils [24,25], it has been shown to increase soil physicochemical properties and have great support for microbial growth [21,26] when acting as organic fertilizer etc. However, its use as a biostimulant in remediation of oily sludge impacted soils has not been given much adequate attention. It is evident that employing MOSC in soil remediation will not only remove the heavy metals, but will increase soil fertility and at the same time solve the problem of waste management. This paper explores the use of plant-derived organic fertilizer, agro-waste from *Moringa Oleifera* seed in biotransformation and removal of heavy metals from petroleum sludge impacted soils.

2. MATERIALS AND METHODS

2.1 Description of Sampling Sites

The Niger Delta is one of the world's largest deltas and makes up approximately 7% of the land area of Nigeria. It has been reported to cover an area of approximately 75,000 km², representing about 7.5 % of Nigeria [1]. The region has about 5284 oil wells and 527 flow stations of crude oil processing with more than 7000 km of oil and gas pipeline traversing across the whole land area (Figure 1). Delta State which is being nicknamed "The Big Heart of the Nation" lies approximately between Longitude 5°00 and 6°.45' East and Latitude 5°00 and 6°.30' North of the equator. It is located in the southern Nigeria with an

area of 17,698 km² (6,833 sq mi) and a population of 4,112,445 as at 2006 [27]. It is made up of 25 Local Government Areas and comprising mainly five major ethnic groups: Urhobo, Isoko, Anioma and Ukwani, Ijaw and Itsekiri. Warri is the biggest commercial city in the state where the refinery is located. The major people in Warri comprise the Urhobos, Ijaws and Itsekiris [28].

The oil spill impacted communities (Itsekiri) are situated between Latitudes 5°30'N and 5°33'N of the Equator and Longitudes 5°45'E of the Prime Meridian, in Warri South Local Government Area of Delta State. Climatologically, the study area has a mean annual rainfall of 3200 mm and mean temperature of about 28°C. Topography is flat, with an average elevation of about 13 m above sea level. The flat and low relief features often encourage flooding after rain events. The drainage pattern is dendritic with major tributaries emptying into the Forcados River. The vegetation of the area is tropical rain forest type, comprising abundant trees and grasses. The soils in the study area exhibits a wide range of colours from milky white through brown to very dark brown; and they vary in types and texture from loamy to sandy and clayey types according to the United States Department of Agriculture (USDA) classification. Warri people are mainly farmers, civil servants, businessmen and women, artisans and entrepreneurs [1]. Due to frequent oil spills from the sludge pits as a result of refining operations from the Warri Refinery and Petrochemical Company (WRPC), the Itsekiri people are faced with the problem of environmental degradation.

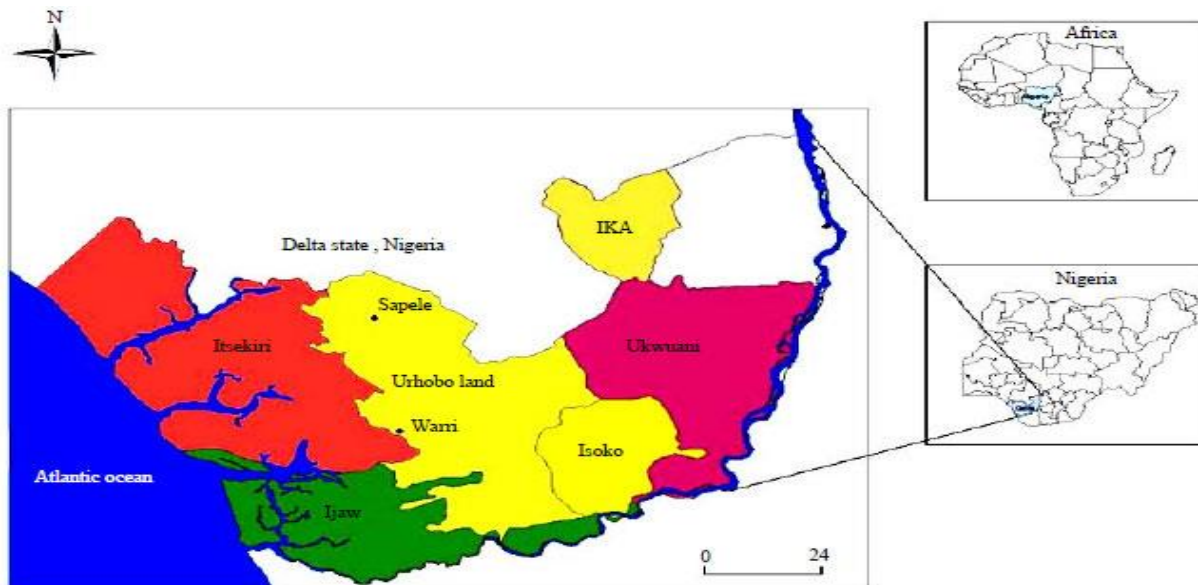


Figure 1: Map of Delta State showing the Study Area

2.2 Sample Collection, Handling and Preservation

US EPA (SW-846) guidelines were applied, using composite sampling for collecting sediment samples where sub-samples were collected from randomly selected locations in an area. Five (5) oily sludge samples were collected from the discharge pit of WRPC with core sampler in a 500 mL wide-mouth glass jar and pooled. Also, fifty (50) soil samples were randomly collected using soil auger from the depth of 0-15 cm from five selected oil-impacted communities (Ubeji – 500 m, Ekpan – 1.5 km, Aja-Etan – 2.5 km, Ifie-Kporo – 3.0 km, Ijala-Ikenren – 3.8 km from

WRPC and were coded A, B, C, D and E respectively) and stored in sealed polythene bags. There were ten (10) replicates for each sampling site and the sub-samples were thoroughly mixed to obtain a representative sample of each. A control sample was also collected 8.5 km away from WRPC. These were stored in well-labeled amber glass bottles with teflon-lined screw cap, held at 4°C immediately in a cooler of ice and transported to the laboratory for pre-treatment and analyses [1]. On reaching the laboratory, stones and debris were removed and the samples were used as arrived for the treatment with agricultural waste from

Moringa Oleifera seed. All analyses were carried out in triplicates to minimise error.

2.3 Preparation of *Moringa Oleifera* Seed Cake (MOSC)

The *Moringa Oleifera* (MO) seed pods were purchased from Kubwa market, FCT, Abuja, Nigeria. They were dehusked and pulverized. The oil in the seed was extracted by hexane using soxhlet extraction method. About 100 g of MO seed powder were poured into an extraction thimble. 1 L of hexane solvent was poured into a round bottom flask. After setting up the soxhlet apparatus, it was heated for 1 hr and the oil was extracted. After extraction, the seed cake was sun dried and pulverized. It was then stored in a clean polyethylene container [29,30].

2.4 Sample Treatment

The samples used for the study were field-moist soil samples, no air-dried material was used. With the soil samples, the agro-waste from moringa seed used as organic fertilizer was added to enhance biodegradation of the contaminant, and the whole mixture was mixed using a mixer. The ratio of organic fertilizer to contaminated soil was one part (20 g) of fertilizer to three parts (60 g) of soil [31,32]. The treated soils were kept under controlled humidity 60 % of field capacity, in the ambient laboratory conditions with temperature (28 ± 4 °C), under subdued light to serve as abiotic factors. The rate of bioremediation was studied as a function of time. Heavy metal removal was measured by monitoring the heavy metal concentrations of the samples after 30 days, after 60 days and after 90 days. The contents of the experiment were manually mixed twice a week to allow aeration and homogenous mixture of the materials [33,34].

At 30 days interval, samples were collected and air-dried for two weeks at ambient temperature, rolled manually with a steel roller, sieved to remove stones and debris. These were further grounded with mortar and pestle until very fine fraction was achieved, it was sieved through a 2-mm stainless steel mesh to get a test sample of <2 mm fraction. Both devices were cleaned after each sample had been processed to avoid cross-contamination. These were properly stored in well-labeled air-tight containers until analysis [4].

2.5 Reagents

All chemicals and reagents were of analytical grade and of highest purity possible. They were supplied by BDH Labs (UK). BDH Chemicals Limited Poole England.

2.6 Heavy Metals Analysis

A test portion of 1.00 g of each soil sample was digested using the conventional aqua regia (3:1, v/v, HCl to HNO₃) digestion procedure. The soil sample was weighed and transferred into the digestion vessel (250 ml glass beaker covered with watch glass). 20 ml of freshly prepared aqua regia mixture was added and mixed by swirling. This was moistened with a little deionized distilled water. Thereafter, the digestion vessel was placed on a heating mantle for 2 h at 110 °C until about 5 ml of digest remained in the flask. The vessel was removed and allowed to cool for 15 min. Then, another 20 ml of freshly prepared aqua regia mixture was added and boiling was repeated until the digest cleared up. After evaporation to near dryness, the sample was allowed to cool and was diluted with 20 ml of 2 % (v/v with H₂O) HNO₃. This was transferred into a 100 ml volumetric flask

after filtering through Whatman no. 42 paper and was made to volume with deionized distilled water. The blank solutions were undergoing the same digestion procedure as that of the sample. All digestions were carried out in triplicates for each sample and the amounts of trace metals recorded as mean value. The extracts were analysed for heavy metals (Cr, Cd, Pb, Ni, Cu, Co, Mn, Fe and Zn) using atomic absorption spectrophotometer (AAS) iCE 3000 Series 3000 at their respective wavelength (357.9, 228.8, 283.3, 232.0, 324.8, 240.7, 279.5, 248.3 and 213.9 nm) according to APHA method [35].

2.7 Preparation of Calibration Standards

For calibration of the instrument, a series of five standard solutions were prepared by serial dilution of the stock standard solutions (1000 mg/l) of the metals to be analyzed.

2.8 Determination of Heavy Metals in Sample Matrices

The digested soil samples were analyzed for the heavy metal concentrations such as Cr, Cd, Pb, Ni, Cu, Co, Mn, Fe and Zn using Atomic Absorption Spectrometer (FAAS) (AAS iCE 3000 Series). Final concentration of element in the samples was calculated as:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L)} \times V}{W} \text{ ---Equ. (1)}$$

Where: V is the final volume of the digested solution (100 ml)
W is the weight of the sample (1.00 g).

2.9 Statistical Analysis

Data analysis involved simple descriptive and univariate summary statistics such as mean, standard deviation and percentage. The heavy metals were the main index for evaluating bioremediation in the different soil samples. Hence, the heavy metals data were subjected to analysis of variance (ANOVA) to compare the variability in metal removal in the different soil samples over time. Two-way ANOVA with replication showed that the metal losses across the different soil samples over time was significant at the 0.01 probability level (significance level of 1%; $p = 0.01$), in accordance with Schmuller (2005). All the statistical analyses were performed using statistical software SPSS Windows version 16.0 [1].

2.10 Toxicity Assessment of Sediment Samples

Some geochemical assessment techniques, including enrichment factor (EF) and geoaccumulation index (I_{geo}), were used in order to determine the levels of metal contamination in the sediments in focus [36]. The computation of enrichment factor (EF) has been adopted to evaluate the impact of anthropogenic activities related to the metal abundance in sediments. According to Moez et al., [37], EF is defined by the following equation:

$$EF = \frac{(C_x/C_{Fe})_{\text{sample}}}{(C_x/C_{Fe})_{\text{crust}}} \text{ ----- (Equ. 2)}$$

whereby

- Fe (iron) is chosen as a natural element of reference
- (C_X/C_{Fe}) sample is the ratio between concentration of the element "X" and that of Fe in the sediment sample
- (C_X/C_{Fe}) crust is the ratio between concentration of the element "X" and that of Fe in unpolluted reference baseline.

According to Birch [38], calculated EF values could be interpreted as follows:

- $EF \leq 1$: no enrichment;
- $1 < EF < 3$: minor enrichment;

- $3 < EF < 5$: moderate enrichment;
- $5 < EF < 10$: moderate-to-severe enrichment;
- $10 < EF < 25$: severe enrichment;
- $25 < EF < 50$: very severe enrichment;
- $EF > 50$: extremely severe enrichment.

The study of geoaccumulation index (I_{geo}) could be relevant in the examination of the contamination level of the sediment samples affected by metals. In Maurizio's [39] view, I_{geo} can be obtained by the following equation:

$$I_{geo} = \text{Log}_2 \left(\frac{C_n}{1.5B_n} \right) \text{----- (Equ. 3)}$$

whereby C_n is the concentration of the metal (n) in sampled and analyzed sediment and B_n is the background concentration of the same metal (n) and the factor 1.5 is the background matrix correction factor due to lithogenic effects [40]

According to Maurizio's [39], calculated I_{geo} values could be interpreted as follows:

$I_{geo} \leq 0$: Uncontaminated

$0 < I_{geo} < 1$: From uncontaminated to moderately contaminated

$1 < I_{geo} < 2$: Moderately contaminated

$2 < I_{geo} < 3$: From moderately contaminated to strongly contaminated

$3 < I_{geo} < 4$: Strongly contaminated

$4 < I_{geo} < 5$: From strongly to extremely contaminated

$I_{geo} > 5$: Extremely contaminated

3. RESULTS AND DISCUSSION

Remediation of heavy metals was carried out by treatment of contaminated soils with MOSC for 90 days as indicated in Tab. 1- 4. From our previous studies, it was observed that the soil samples were highly contaminated with all the studied metals except Ni which was found only in the sludge and site A. Also, the concentrations of heavy metals were seen to increase as a result of the proximity of each site to the refinery except for Cr in site E and Co in site D [1]. After the first 30 days of remediation studies, the concentrations of Co and Fe reduced to values lower than their control values at day 1 (10.90 ± 0.03 mg/kg and 6057.13 ± 2.12 mg/kg respectively) in all the studied sites (Tables 1 and 2) and the reduction was statistically significant ($p < 0.01$) [1]. The concentration of Cd was lower than the control value at day 1 (0.60 ± 1.13 mg/kg) only in site E (0.58 ± 0.00 mg/kg), however, this reduction was not statistically significant ($p > 0.01$). The concentrations of other metals were still higher than their control values at day 1 after the first 30 days of treatment, however, remediation was feasible for all the heavy metals in all the sites.

At 60 days of remediation studies, in addition to Co, Fe and Cd, the concentrations of Cu was seen to decrease to values lower than its control value at day 1 (6.69 ± 0.01 mg/kg) except in sites A and B while Zn concentration was lower than its control value at day 1 (245.85 ± 0.03 mg/kg) in site E (212.39 ± 0.17 mg/kg) only. The concentrations of all the metals were observed to decline consistently, but the reduction was not statistically significant ($p > 0.01$) as compared to their control counterparts at day 1 (Table 1 and 3).

Table 1: Heavy Metals concentrations of the Samples at day 1 (mg/kg)

HEAVY METALS	SITE A	SITE B	SITE C	SITE D	SITE E	CONTROL
Cr	166.79±0.27	116.71±0.21	97.86±0.05	83.55±0.10	90.88±0.13	23.89±0.10
Cd	3.56±0.06	2.26±0.02	1.82±1.07	1.57±0.36	1.10±2.83	0.60±1.13
Pb	157.64±0.16	144.94±0.15	126.18±0.12	119.54±0.16	112.98±0.11	28.49±0.07
Ni	29.64±0.03	ND	ND	ND	ND	ND
Cu	26.10±0.01	20.22±0.02	19.99±0.01	14.27±0.08	11.33±0.08	6.69±0.01
Co	22.90±0.03	11.56±0.02	11.46±0.02	13.60±0.02	11.96±0.03	10.90±0.03
Mn	8004.22±0.30	7945.51±0.66	6111.75±1.25	5760.99±1.01	5628.10±0.23	2011.07±0.33
Fe	10313.45±2.31	10061.91±1.96	9484.92±0.71	8567.82±1.41	7429.79±1.44	6057.13±2.12
Zn	916.33±0.23	673.92±0.15	604.93±0.11	577.22±0.15	515.44±0.20	245.85±0.03

ND = not detected

Table 2: Heavy Metals Concentrations of the Samples after 30 days (mg/kg)

HEAVY METALS	SITE A	SITE B	SITE C	SITE D	SITE E	CONTROL
Cr	79.89±0.04	58.17±0.07	52.91±0.05	45.10±0.07	45.20±0.04	9.84±0.02
Cd	1.64±0.00	1.60±0.01	1.05±0.00	0.88±0.00	0.58±0.00	0.33±0.00
Pb	78.94±0.03	72.31±0.03	63.09±0.02	58.92±0.03	57.52±0.03	13.28±0.01
Ni	15.63±0.02	ND	ND	ND	ND	ND
Cu	12.51±0.02	12.24±0.02	11.61±0.02	8.89±0.02	7.41±0.02	3.17±0.01
Co	8.02±0.04	5.65±0.02	4.99±0.02	5.51±0.01	4.67±0.01	3.46±0.02
Mn	4230.68±1.45	4107.60±1.07	3259.71±1.46	3011.44±0.63	2925.58±0.45	1033.01±0.47
Fe	5521.56±0.45	5229.89±0.42	4943.89±0.00	4474.15±0.52	3866.32±0.36	3308.98±0.80
Zn	509.94±0.30	373.92±0.37	355.36±0.60	315.76±0.27	300.33±0.32	165.64±0.51

ND = not detected

Table 3: Heavy Metals Concentrations of the Samples after 60 days (mg/kg)

HEAVY METALS	SITE A	SITE B	SITE C	SITE D	SITE E	CONTROL
Cr	60.99±0.02	48.93±0.08	34.50±0.08	30.63±0.03	27.25±0.10	5.52±0.02
Cd	1.05±0.00	1.02±0.00	0.86±0.00	0.74±0.00	0.44±0.00	0.21±0.00
Pb	57.60±0.06	54.09±0.04	48.30±0.02	44.00±0.03	35.13±0.13	7.80±0.03
Ni	10.75±0.01	ND	ND	ND	ND	ND
Cu	8.79±0.03	6.82±0.03	5.04±0.03	4.96±0.02	3.96±0.01	2.28±0.01
Co	6.52±0.03	4.50±0.02	3.99±0.02	3.98±0.02	4.08±0.00	2.82±0.01
Mn	3216.24±2.38	3138.32±2.98	2511.85±0.60	2397.95±1.06	2335.26±0.82	829.55±0.47
Fe	4538.56±1.36	4314.51±1.51	4117.04±1.65	3705.64±1.63	3246.36±1.96	2766.64±1.91
Zn	405.05±0.13	301.07±0.28	268.73±0.22	252.60±0.18	212.39±0.17	92.78±0.17

ND = not detected

Table 4: Heavy Metals Concentrations of the Samples after 90 days (mg/kg)

HEAVY METALS	SITE A	SITE B	SITE C	SITE D	SITE E	CONTROL
Cr	33.02±0.04	22.98±0.03	21.43±0.02	16.68±0.03	17.65±0.03	3.98±0.00
Cd	0.84±0.00	0.59±0.00	0.52±0.00	0.42±0.00	0.34±0.00	0.21±0.00
Pb	39.45±0.05	35.87±0.04	30.91±0.03	27.70±0.02	26.46±0.01	5.94±0.00
Ni	6.15±0.01	ND	ND	ND	ND	ND
Cu	6.03±0.01	4.61±0.01	4.36±0.01	3.35±0.00	2.45±0.01	1.56±0.00
Co	4.62±0.01	3.92±0.01	2.87±0.01	2.94±0.00	4.04±0.00	2.01±0.00
Mn	1883.76±0.91	1832.06±1.35	1439.10±0.43	1397.30±0.36	1338.20±0.55	459.19±0.53
Fe	2465.58±1.28	2368.48±0.75	2238.98±0.87	2017.42±0.69	1709.01±1.12	1461.96±0.74
Zn	207.03±0.29	167.52±0.08	152.32±0.09	67.42±0.76	121.35±0.11	74.22±0.19

ND = not detected

At 90 days which was the end of the treatability studies, the concentrations of Cr and Cd were observed to reduce to values lower than their control values at day 1 (23.89±0.10 mg/kg and 0.60±1.13 mg/kg respectively) in all the sites except in site A which was very close to the refinery. The concentration of Pb compared to its control value at day 1 (28.49±0.07 mg/kg) was seen to decrease only in sites D and E which were farther away from the refinery, while sites A, B and C still had higher values of Pb though remediation was feasible (Table 4). Ni was detected only in site A and there was a consistent reduction of Ni throughout the treatability studies. This is in variance with previous studies carried out by Rakesh and Raju [41] who reported that addition of organic amendment decreased the solubility of As, Cd, Cu, Mo, and Pb while the solubility of Ni and Zn was not changed. The variation observed maybe due to the different concentration and composition of petrochemicals applied in the research. The concentrations of Cu, Co, Mn, Fe and Zn demonstrated reduction to values lower than their control counterparts at day 1 indicating that remediation was statistically significant ($p < 0.01$) (Table 4). Generally, there was significant decrease of all the heavy metals in all the sites from day 1 to day 90 which was the end of the treatability studies. Specifically, site A which had the highest metal concentration also showed the highest metal removal compared to the control which was also treated with the agro-waste. This may be attributed to the nutrient imbalance in the control site since it was not contaminated. This is in agreement with Rajendran *et al.*, [42] who reported that microbial interactions with small quantities of metals do not exert a major impact on metal distribution in the environment, whereas interaction with larger quantities are required in energy metabolism to have noticeable impact. The presence of one heavy metal may affect the availability of another in the soil and hence plant. This shows that, antagonistic and synergistic behaviours exist among heavy metals. Sana *et al.*, [43] reported that the inhibitory effect of Mn on the total amount of mineralized C was antagonized by the presence of Cd. In

contrast, Cu was reported to increase the toxicity of Zn in spring barley [44]. This implies that the interrelationship between heavy metals is quite complex; thus more research is needed in this area.

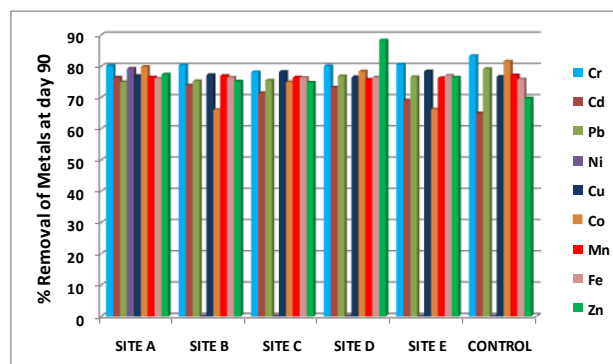


Fig. 2: Percentage Removal of Heavy Metals at day 90

The percentage removal of the metals conducted at the end of the treatability studies is depicted in Fig. 2. It was observed that the percentage removal of Cr, Pb, Co and Mn were higher in their control samples (83.34 %, 79.15 %, 81.56 % and 77.17 % respectively) than the contaminated sites while Cd, Fe and Zn had the least percentage removal (65.00 %, 75.86 % and 69.81 % respectively) at their control sites. From the graph, it was revealed that Cr had relatively the highest percentage removal except in site D, however, the effect of its residual concentration is not yet known but can be determined by employing some metal indicators like Enrichment Factor (EF) and Geoaccumulation Index (Igeo). In general, the percentage removal of all the heavy metals was between 65.00 % and 88.32 % which showed a successful bioremediation. According to Guarino *et al.*, [45], a bioremediation activity has successfully occurs when 65 % or more heavy metals were removed from the system.

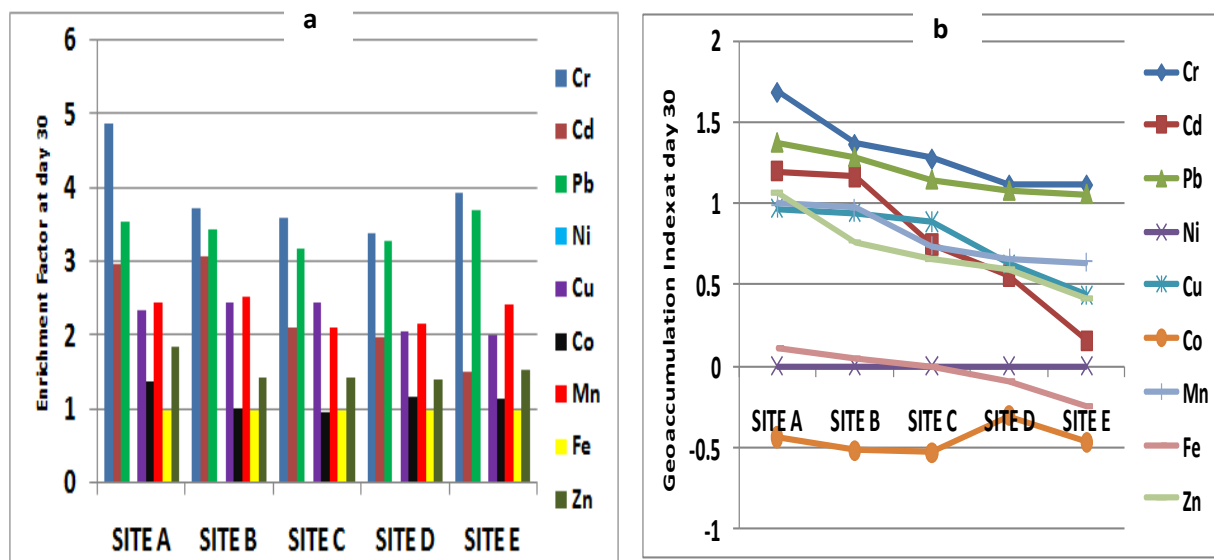


Fig. 3: EF and Igeo of Heavy Metals at day 30 (mg/kg)

Table 5: (*Igeo*) classification and ranges of heavy metals in the Samples at day 30 (mg/kg)

<i>Igeo</i>	Classifications	Level of Contamination	Cr	Cd	Pb	Ni	Cu	Co	Mn	Fe	Zn
$Igeo \leq 0$	0	Uncontaminated	-	-	-	Sites A, B, C, D, E	-	Sites A, B, C, D, E	-	Sites C, D, E	-
$0 < Igeo < 1$	1	From Uncontaminated to moderately contaminated	-	Sites C, D, E	-	-	Sites A, B, C, D, E	-	Sites B, C, D, E	Sites A, B	Sites B, C, D, E
$1 < Igeo < 2$	2	Moderately contaminated	Sites A, B, C, D, E	Sites A, B	Sites A, B, C, D, E	-	-	-	Sites A	-	Site A
$2 < Igeo < 3$	3	From moderately contaminated to strongly contaminated	-	-	-	-	-	-	-	-	-
$3 < Igeo < 4$	4	Strongly contaminated	-	-	-	-	-	-	-	-	-
$4 < Igeo < 5$	5	From strongly to extremely contaminated	-	-	-	-	-	-	-	-	-
$Igeo > 5$	6	Extremely contaminated	-	-	-	-	-	-	-	-	-

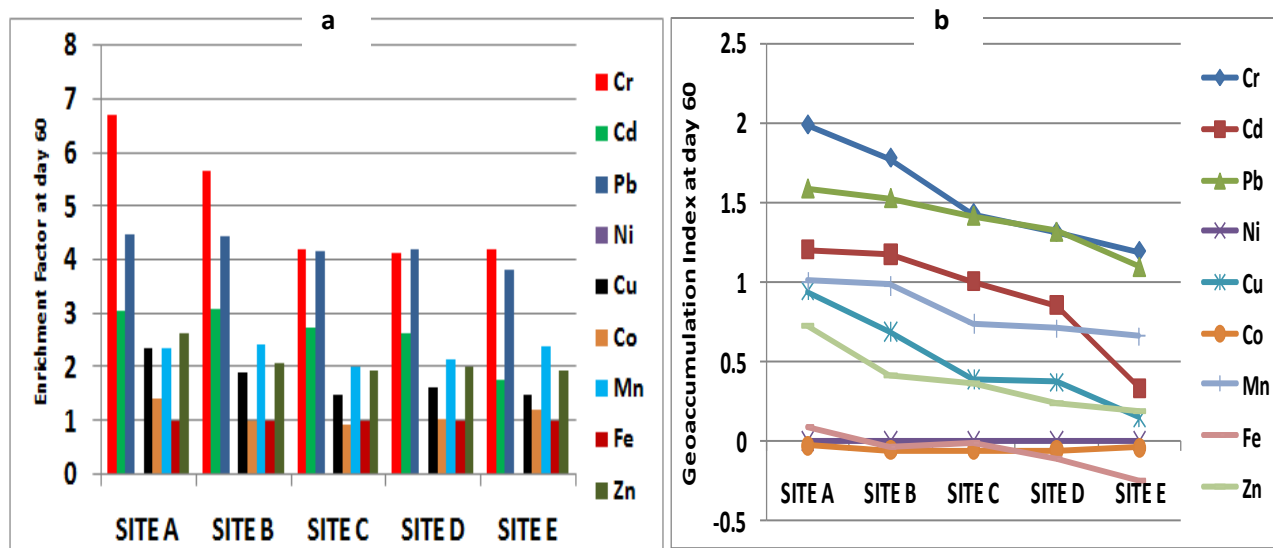


Fig. 4: EF and Igeo of Heavy Metals at day 60 (mg/kg)

Table 6: (*Igeo*) classification and ranges of heavy metals in the Samples at day 60 (mg/kg)

<i>Igeo</i>	Classification	Level of Contamination	Cr	Cd	Pb	Ni	Cu	Co	Mn	Fe	Zn
$Igeo \leq 0$	0	Uncontaminated	-	-	-	Sites A, B, C, D, E	-	Sites A, B, C, D, E	-	Sites B, C, D, E	-
$0 < Igeo < 1$	1	From Uncontaminated to moderately contaminated	-	Sites D, E	-	-	Sites A, B, C, D, E	-	Sites B, C, D, E	Site A	Sites A, B, C, D, E
$1 < Igeo < 2$	2	Moderately contaminated	Sites A, B, C, D, E	Sites A, B, C	Sites A, B, C, D, E	-	-	-	Site A	-	-
$2 < Igeo < 3$	3	From moderately contaminated to strongly contaminated	-	-	-	-	-	-	-	-	-
$3 < Igeo < 4$	4	Strongly contaminated	-	-	-	-	-	-	-	-	-
$4 < Igeo < 5$	5	From strongly to extremely contaminated	-	-	-	-	-	-	-	-	-
$Igeo > 5$	6	Extremely contaminated	-	-	-	-	-	-	-	-	-

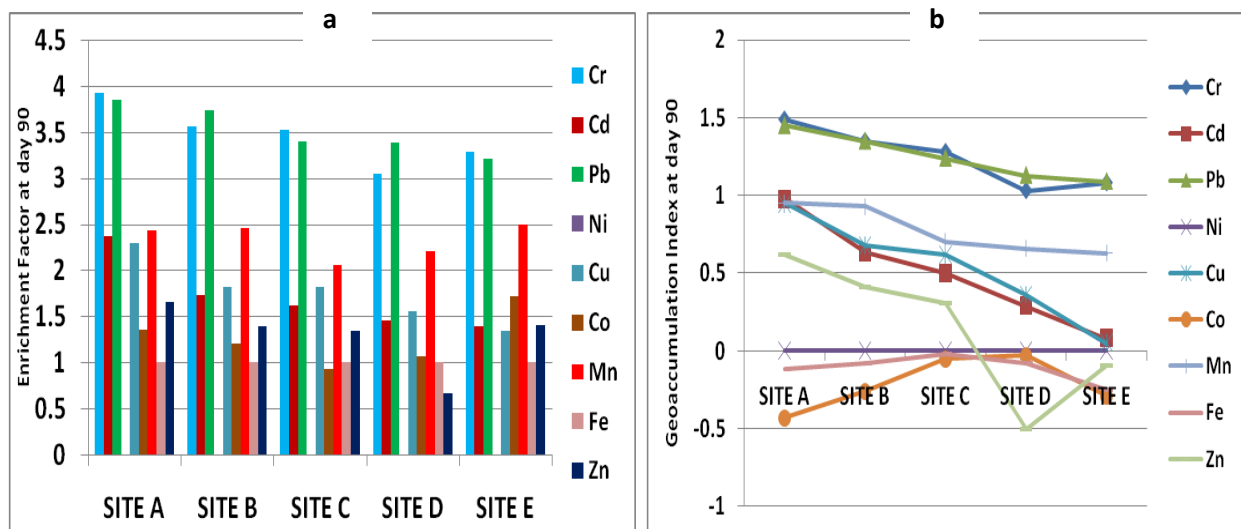


Fig. 5: EF and Igeo of Heavy Metals at day 90 (mg/kg)

Table 7: (Igeo) classification and ranges of heavy metals in the Samples at day 90 (mg/kg)

Igeo	Classification	Level of Contamination	Cr	Cd	Pb	Ni	Cu	Co	Mn	Fe	Zn
$I_{geo} \leq 0$	0	Uncontaminated	-	-	-	Sites A, B, C, D, E	-	Sites A, B, C, D, E	-	Sites A, B, C, D, E	Sites D, E
$0 < I_{geo} < 1$	1	From Uncontaminated to moderately contaminated	-	Sites A, B, C, D, E	-	-	Sites A, B, C, D, E	-	Sites A, B, C, D, E	-	Sites A, B, C
$1 < I_{geo} < 2$	2	Moderately contaminated	Sites A, B, C, D, E	-	Sites A, B, C, D, E	-	-	-	-	-	-
$2 < I_{geo} < 3$	3	From moderately contaminated to strongly contaminated	-	-	-	-	-	-	-	-	-
$3 < I_{geo} < 4$	4	Strongly contaminated	-	-	-	-	-	-	-	-	-
$4 < I_{geo} < 5$	5	From strongly to extremely contaminated	-	-	-	-	-	-	-	-	-
$I_{geo} > 5$	6	Extremely contaminated	-	-	-	-	-	-	-	-	-

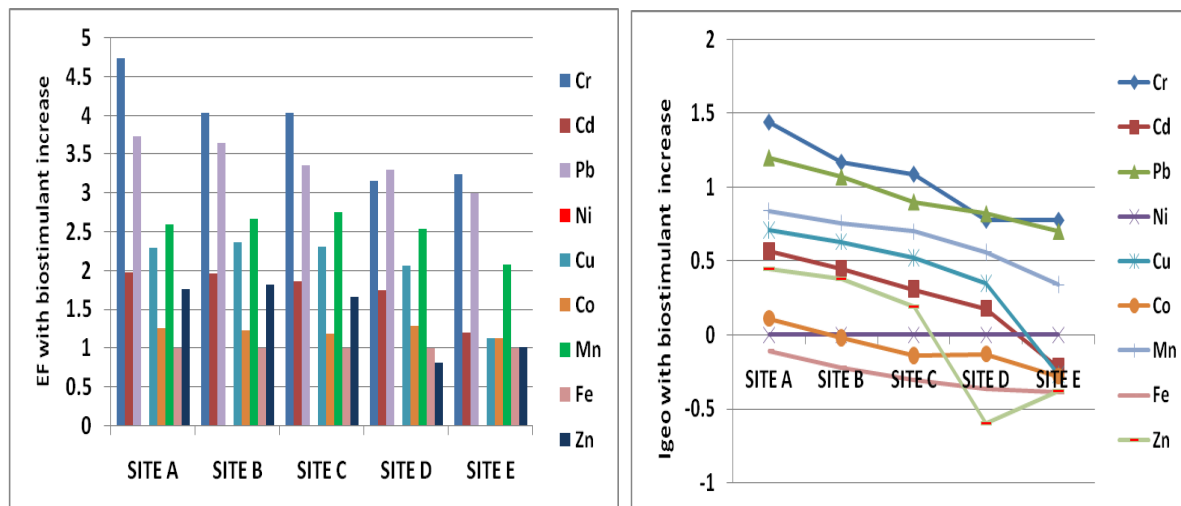


Fig. 6: EF and Igeo of Heavy Metals with increment of MOSC (mg/kg)

The Enrichment Factor (EF) and Geoaccumulation Index (Igeo) in metals are indicators used to assess the presence and intensity of anthropogenic contaminant deposition on surface soil. From the EF depicted in Figs. 3a, 4a, and 5a, the average results demonstrated that, using Fe as a reference concentration, the EF was relatively high. EF values greater than 1 suggest that the sources are more likely to be anthropogenic according to Ghrefat *et al.*, [46] and Selena *et al.*, [47]. From Fig. 3a, after the first 30 days of remediation, the computed EF indicated that there was no enrichment of Ni and Fe in all the studied sites, also there was no enrichment of Co in site C ($EF \leq 1$). Sites A, C, D and E showed minor enrichment of Cd, Cu, Co, Mn and Zn ($1 < EF < 3$) and moderate enrichment of Cr and Pb ($3 < EF < 5$) while Site B demonstrated minor enrichment of Cu, Co, Mn, Fe and Zn ($1 < EF < 3$) and moderate enrichment of Cr, Cd and Pb ($3 < EF < 5$). After the first 60 days of remediation studies (Fig. 4a), the EF of Cr, Pb and Zn were observed to increase which was contrary to expectation though in agreement with previous studies by Moez *et al.*, [37]. This could be as a result of the rate of removal of Fe from the control sample, and also the depletion of available nutrients by the soil microbes. From Fig. 3, there was no enrichment of Ni and Fe in all the studied sites, also there was no enrichment of Co in site C ($EF \leq 1$). Sites A and B showed minor enrichment of Cu, Co, Mn and Zn ($1 < EF < 3$), moderate enrichment of Cd and Pb ($3 < EF < 5$) and moderate-to-severe enrichment of Cr ($5 < EF < 10$) while sites C, D and E demonstrated minor enrichment of Cd, Cu, Co, Mn and Zn ($1 < EF < 3$) and moderate enrichment of Cr and Pb ($3 < EF < 5$). At the end of the remediation studies which was 90 days, there was no enrichment of Ni and Fe in all the sites, also there was no enrichment of Co and Zn in sites C and D respectively ($EF \leq 1$) (Fig. 5a). All the studied sites revealed minor enrichment of Cd, Cu, Co, Mn and Zn ($1 < EF < 3$) and moderate enrichment of Cr and Pb ($3 < EF < 5$). The difference in EF values for the different metals in the surface sediments may be due to the difference in the magnitude of input for each metal in the sediment and/or the difference in the removal rate of each metal from the sediment.

The values of EF in the study area were similar to those found by Moez *et al.*, [37] and were higher than the EF recorded by Abraham and Parker [48] in Tamaki Al-Arab Sediment.

The geoaccumulation index (Igeo) computations for the sediment samples after remediation are depicted in Figs. 3b, 4b and 5b. Summary and interpretation of the Igeo values for individual elements in the five studied sites are presented in Tables 5, 6 and 7. From Fig. 3b, after the first 30 days of remediation studies, the computed Igeo demonstrated that all the sites were decontaminated of Ni and Co, likewise, sites C, D and E were also decontaminated of Fe ($I_{geo} \leq 0$). The Igeo also showed that sites C, D and E were uncontaminated to moderately contaminated with respect to Cd, Cu, Mn and Zn, likewise in site A with respect to Cu and Fe and site B with respect to Cu, Mn, Fe and Zn ($0 < I_{geo} < 1$). Further, the computed Igeo revealed that all the sites were moderately contaminated with Cr and Pb, as well as in site A with respect to Cd, Mn and Zn, and site B with respect to Cd ($1 < I_{geo} < 2$). At the end of 60 days of treatment, a similar Igeo trend was observed except that site B was decontaminated of Fe ($I_{geo} \leq 0$) (Fig. 4b). At the end of the treatability studies, the computed Igeo revealed that all the studied sites were decontaminated of Ni, Co and Fe, likewise sites D and E with respect to Zn ($I_{geo} \leq 0$). The Igeo also demonstrated that all the sites were uncontaminated to moderately contaminated with respect to Cd, Cu and Mn, as well as sites A, B and C with respect to Zn ($0 < I_{geo} < 1$). Further, the index of geoaccumulation revealed that at the end, all the studied sites were still moderately contaminated with Cr and Pb ($1 < I_{geo} < 2$) (Fig. 5b). Based on the classification system proposed for Igeo factors, the average Igeo class is 0 – 2 indicating uncontaminated to moderately contaminated levels at the end of the study.

The EF and Igeo of Pb and Cr at the end of the study prompted further research on varying the proportion of soil to MOSC in the ratio of 3:2 and monitored it for another 30 days. It was observed that the concentrations of the metals drastically reduced compared to values at day 1 but the computed EF showed no significant difference from the result obtained at the end of the

study period (Fig. 6). The computed Igeo further demonstrated that there was no significant difference from the Igeo result observed at the end of the study period except that in addition, site E was decontaminated with respect to Cd and Cu ($I_{geo} \leq 0$) (Fig. 6). The Igeo also revealed that sites D and E were uncontaminated to moderately contaminated with respect to Cr and Pb as well as site C with respect to Pb ($0 < I_{geo} < 1$) while sites A and B still showed moderate contamination of Cr and Pb likewise site C with respect to Cr ($1 < I_{geo} < 2$) (Fig. 6). This indicated that there could be potential for improvement with time by using higher concentration of MOSC. However, the observed improvement was not proven to be significant. Addition of higher mix ratio did not produce a corresponding increase in the removal of residual metals as expected. In other words, functional or genetic diversity of soil microorganisms was not greatly affected by biostimulant increment, probably because of the reduction of competition among microbial population due to the increase in resources, as nutrient contents in amended soils. It has been reported that excessive nutrient availability can also inhibit the bioremediation of contaminants [43,49,50]. Bioremediation emerges as a simple and eco-friendly technology over the conventional methods for remediation of soil due to the complexity and environmental hazards associated with them.

4. CONCLUSION

Bioremediation is measured to be very safe and obliging technology as it depends on microbes that occur naturally in the soil and pose no hazard to environment and the people living in the area. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. There is no doubt that bioremediation is in the process of paving a way to greener pastures. Regardless of which aspect of bioremediation that is used, this technology offers an efficient and cost effective way to treat contaminated soil. After 90 days of treatment, it was observed that the studied heavy metals except Cr and Pb biodegraded to levels that are no more toxic to the environment. The computed EF and Igeo of Pb and Cr at the end of the study prompted further research on varying the proportion of soil to MOSC in the ratio of 3:2 and monitored it for another 30 days. It was observed that the concentrations of the metals drastically reduced compared to values at day 1 but the computed EF and Igeo showed no significant difference from the result obtained at the end of the study period. Addition of higher mix ratio did not produce a corresponding increase in the removal of residual metals as expected, probably because of the reduction of competition among microbial population due to the increase in resources, as nutrient contents in amended soils. This research has shown that bioremediation technology though being simple and eco-friendly, is not without some limitations; several microorganisms cannot really break toxic metals like Pb and Cr into harmless metabolites, and these have inhibitory effects on microbial activity. However, bioremediation using organic amendment have been confirmed to have the ability to transform most heavy metals to less toxic forms. From this study, it can be concluded that MOSC has high potential of removing heavy metals from a contaminated soil. The sanitization of these natural resources is important for the preservation of nature and environment using bioremediation process.

However, this research has shown that bioremediation technology also has limitations; several microorganisms cannot really break toxic metals like Pb and Cr into harmless metabolites, and these have inhibitory effects on microbial activity. Modification in the outer membrane proteins of bacteria with potential bioremediation properties for improving metal binding abilities is the likely way to enhance their capacity for biotransformation of toxic metals in all the possible adverse/stress conditions and multiple-heavy-metal-contaminated environments.

Significant difference between the concentrations of heavy metals observed in this study proved that, MOSC have an undiscovered and unexplored potential for the remediation of metals in the soil. Variance in the removal of heavy metal in different sites could be attributed to the fact that some soil microbe tend to be more specific and sensitive to one metal but have higher tolerance to other metals. Microbiological processes in the soil can either solubilize metals, thereby increasing their bioavailability and potential toxicity, or immobilize them, hence reduce the bioavailability of these metals. These biotransformations are important components of biogeochemical cycles of metals exploited in bioremediation of metal contaminated soils.

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