SHIFT IN POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METAL CONCENTRATIONS OF A CRUDE OIL-POLLUTED SOIL UNDERGOING LABORATORY-SCALE BIOREMEDIATION

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ABSTRACT

The shift in polycyclic aromatic hydrocarbons (PAHs) and heavy metal concentrations during the laboratory-scale bioremediation of crude oil-polluted soil from the K-Dere, Ogoni land, Nigeria was studied. Crude oil-polluted soil samples were collected and processed, and bioremediation experimental treatment units were set up to monitor the changes in PAHs and heavy metal concentrations within 40 days using different treatment options. The treatment options consist of four experimental units, which include sample A (polluted soil only), sample B (polluted soil and bacterial consortium), sample C (polluted soil and NPK) and sample D (polluted soil and cow dung). PAHs were analysed using the Gas Chromatography-Flame Ionization Detection, while the heavy metals were analysed using the Atomic Absorption Spectrophotometer. There was an overall reduction in PAH and heavy metal concentrations after treatment. A PAH loss of 12.30% was recorded in sample A, 60.82% in sample B, 44.75% in sample C and 21.03% in sample D. Sample B, which had the bacterial consortium, experienced the highest PAH reduction, while sample A recorded the lowest PAH reduction. Lead, nickel and chromium concentrations recorded slight decreases of 15.20, 16.50 and 19.40%, respectively. Sample D recorded the highest reduction in zinc concentration of 32.00%, while sample B recorded the highest reduction in copper concentration on day 20. Cadmium, iron and copper concentrations significantly reduced in sample B on day 40. This study has further revealed the need to explore Indigenous bacterial consortium and other bioremediation approaches to recover PAH and heavy metal-impacted ecosystems.

Keywords: Polycyclic aromatic hydrocarbon, Heavy metals, Bacteria, Bioremediation, Treatment, Crude oil, Pollution

INTRODUCTION

The recovery of crude oil-contaminated soils by bioremediation is a sustainable and economically viable approach that utilizes microorganisms to degrade pollutants in crude oil-contaminated soils. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are known pollutants that pose serious environmental and health challenges today (Ali *et al.*, 2022). On the other hand, petroleum exploration, development and production activities have localized negative and severe effects on the environment (Brown and Tari, 2015). Environmental contaminations, bad human health consequences, negative influence on the nation's economy and the deterioration of the environment have all resulted from the discharge of crude oil-derived hydrocarbon and petroleum-derived waste streams into the ecosystem (Johnston *et al.*, 2019).

The large quantities of oil that go into the environment, particularly farmlands and rivers, make the clean-up of oil-contaminated sites urgently necessary. Current mechanical techniques usually only recover 10 – 15% of the hydrocarbons following major spills, often leaving the medium in a terrible and undesirable state (Abu and Dike, 2008). An essential aspect of effectively rehabilitating polluted areas is comprehending the dynamics of PAHs and heavy metal concentrations during bioremediation processes. The potential of using bacteria to break down PAHs has recently gained increasing research focus as several bacterial groups have shown their ability to mineralize hydrocarbon components, converting them to degradable organic substances, CO_2 and H_2O (Chikere and Ekwuabu, 2014). Biodegradation is the key hydrocarbon removal process. This process can be controlled by monitoring the physico-chemistry of the hydrocarbons, ecological factors, bioavailability, and the activity of metabolically functional microorganisms (Stroud *et al*., 2007).

Multiple studies have documented the efficacy of bioremediation in decreasing the levels of PAHs and heavy metals in soils polluted with crude oil (Pande *et al*., 2022; Ansari *et al*., 2023). Nevertheless, the precise processes that control the fate and transport of PAHs during bioremediation processes are still a subject of ongoing investigation.

Considerable research has been conducted on microbial communities' biodegradation of PAHs. A variety of bacteria like *Pseudomonas*, *Bacillus*, *Klebsiella*, *Alcanivorax*, *Alcaligenes*, *Acinetobacter*, *Achromobacter*, *Collimonas*, *Gordonia*, *Micrococcus*, *Rhodococcus,* etc., have been shown to degrade PAHs effectively via enzymatic mechanisms (Haritash and Kaushik, 2009; Abdusalam *et al*., 2011). Microorganisms actively accumulate and immobilize heavy metals, which greatly reduces their bioavailability in soils (Abo-Alkasem *et al.,* 2023). The dynamic ecological interactions between bacterial populations and pollutants in the soil matrix significantly impact the effectiveness of bioremediation techniques (Romantschuk *et al*., 2023).

This study targets monitoring, analysing, and revealing the shifts in PAHs and heavy metal concentrations in soil contaminated with crude oil during laboratory-scale bioremediation using different treatment options. By monitoring bacterial community dynamics and the degradation profile of contaminants, this study will provide valuable insights into the mechanisms responsible for the changes in concentrations and the need for improvement of bioremediation techniques for contaminated soil treatment will be addressed.

Ultimately, studying the change in PAHs and heavy metal concentrations during laboratory-scale bioremediation of soil contaminated with crude oil is crucial for enhancing our knowledge of how contaminants are deposited and transported in polluted ecosystems. By clarifying the relationship between bacterial populations and pollutants, this study will provide valuable insights for improving novel bioremediation approaches to tackle the problems associated with soil pollution hazards. This study is aimed at investigating the variations in PAHs and heavy metal concentrations in soil contaminated with crude oil that is being subjected to laboratory-scale bioremediation.

MATERIALS AND METHODS

Site Description: Samples were taken from crude oil polluted sites in K-Dere community of Ogoni land within April and June 2024. These sites have experienced prolonged pollution from oil industry facilities and exploration. Ogoni land covers over 1000 km^2 in Rivers State, South-South, Niger Delta, Nigeria. The global positioning system (GPS) was used to compute the coordinates of the sample sites. The coordinates are K-Dere 1 N $4^0 5^0 45' 59''$ E $07^0 15' 0''$and K-Dere II N $04^0 15' 0''$ E $07^0 16' 0''$.

Soil Sample Collection, Processing and Source of Nutrient Amendment: Crude oil-polluted soil was collected at 0 – 100 cm depths using a soil auger from different points of each site and made into composite samples (homogenous mixture), then put in sterile dark polyethene bags and transported to the laboratory for analysis (Maduwuba, 2024a). The

soil sample was processed by slightly modifying the method of Suja *et al.* (2014) and Maduwuba (2024a). The soil sample was dried in the oven at 40^0C for 3 hours and then sieved with a 2 mm mesh sieve to remove undesired particles before analysis.

The cow dung was collected from a commercial livestock farm at Obinze community, Owerri West, Imo State, Nigeria. The inorganic fertiliser (NPK 15:15:15) was procured from Notore Fertilizer Company Limited, Notore Industrial Complex, Onne, Rivers State, Nigeria.

Bioremediation Setup: The bioremediation setup consists of four (4) experimental units in triplicates as illustrated in Table 1.

Table 1: Experimental setup for the determination of the levels of polycyclic aromatic hydrocarbons and heavy metal in a crude oil-polluted soil undergoing laboratory-scale bioremediation

Groups	Treatment	Design setup
A	1000 g polluted soil only	Negative control
B	1000 g polluted soil + 100 ml bacterial consortium	Positive control
C	1000 g polluted soil + 100 g NPK	Test treatment I
D	1000 g polluted soil + 100 g cow dung	Test treatment II

These setups were monitored for PAH and heavy metals levels for 40 days. Data collected from the various treatments on days 1, 20 and 40 as the experiment progressed during the study were compared.

Gas Chromatography Flame-Ionization Detection for Polycyclic Aromatic Hydrocarbons: Residual concentrations of PAHs were extracted from the samples using the procedure outlined by USEPA (2007). The soil samples of 5 g each were mixed with drying agent anhydrous sodium sulphate and volatile surrogate 2-bromobenzofluoride before extraction using equal ratios of n-hexane/DCM to extract PAHs by cold extraction. Gas chromatography-flame ionization detector (GC-FID) system HP5890 Series II USA was used for quantification/analysis with hypodermic syringes. Helium was used as the carrier gas at a flow rate of 14.81 psi and hydrogen and air as ignition gases at a flow rate of 30 psi (Aigberua, 2019). The split and split-less injection technique was employed. A six-point was prepared for the calibration curve using the stock standard solution at the beginning of sample preparation with calibration levels of 2, 5, 10, 20, 50 and 100 ppm (Aigberua, 2020).

Heavy Metal Analysis of Samples: Soil samples were prepared for heavy metal content analysis by digesting 10 g of air-dried samples with 0.2M nitric acid (HNO_3^-) acid solution for 1 hour in a digestion flask over a hot plate. The digestion was done without letting the acid dry by constantly topping it with the acid solution until digestion was concluded. The digest was filtered through a Whatman filter paper (No. 1), and the volume was made up to 100 ml by flushing the sample with distilled water. The filtrates were analyzed for their heavy metals (lead, zinc, copper, iron, nickel, chromium, and cadmium) concentrations using the atomic absorption Spectrophotometer (AAS) GBC 908PBMT, Australia.

Statistical Analysis: The data obtained from this study were subjected to a one-way analysis of variance (ANOVA). The means of the variables were considered statistically significant at 95% confidence interval ($p<0.05$). The results were expressed in mean ± SEM and presented in bar graphs.

RESULTS

The polycyclic aromatic hydrocarbon analysis showed an overall reduction in PAH concentrations throughout the treatment. For sample A, PAH concentration reduced from 63.58 ± 0.02 to 60.75 ± 0.01 mg/kg and then to 55.75 ± 0.04 mg/kg, indicating a percentage reduction of 4.44% and 12.30% on days 20 and 40 respectively. In Sample B, PAH concentration reduced from 63.58 ± 0.02 to 35.78 ± 0.01 mg/kg and then to 24.91 ± 0.32 mg/kg, indicating a percentage reduction of 43.72% and 60.82% on days 20 and 40 respectively. In Sample C, PAH concentration reduced from 63.58 ± 0.02 to 38.91 ± 0.00 mg/kg and then to 35.24 ± 0.07 mg/kg, indicating a percentage reduction of 38.79% and 44.57% on days 20 and 40

respectively. Also, for sample D, PAH concentration reduced from 63.58 ± 0.02 to 55.18 ± 0.00 mg/kg and then to 50.20 ± 0.04 mg/kg, indicating a percentage reduction of 13.20% and 21.03% on days 20 and 40 respectively. Sample B showed the highest percentage loss of PAH, while sample A had the lowest percentage loss of PAH as represented in Figure 1.

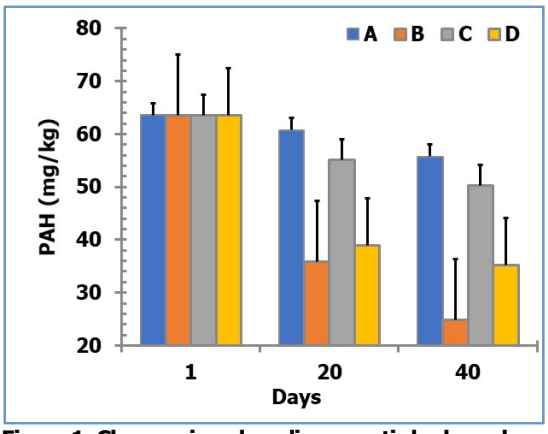

Figure 1: Changes in polycyclic aromatic hydrocarbon concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

There was an overall decrease in lead concentration with time in the different treatments as shown in Figure 2.

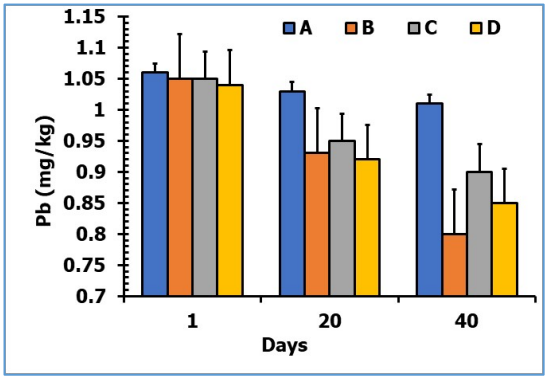

Figure 2: Changes in lead concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

Sample A had lead concentrations of 1.06 ± 0.03, 1.03 ± 0.02 and 1.01 ± 0.01 mg/kg on days 1, 20 and 40, respectively. Sample B had 1.05 ± 0.02, 0.93 ± 0.04 and 0.80 ± 0.04 mg/kg on days 1, 20 and 40 respectively. Sample C had 1.04 ± 0.01, 0.92 ± 0.01 and 0.85 ± 0.01 mg/kg on days 1, 20 and 40, respectively, while sample D was 1.05 ± 0.01 mg/kg, 0.95 ± 0.02 mg/kg and 0.90 ± 0.00 mg/kg on days 1, 20 and 40 respectively. Sample A had the highest lead concentration within the 40-day treatment period, while sample B had the lowest lead concentration on day 40.

Zinc concentration decreased within the treatment periods. Sample A had zinc concentrations of 4.52 ± 0.01, 4.20 ± 0.22 and 4.05 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample B had 4.50 ± 0.04, 3.50 ± 0.22 and 2.81 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample C had 4.42 ± 0.01, 2.60 ± 0.08 and 2.14 ± 0.02 mg/kg on days 1, 20 and 40 respectively, while sample D had zinc concentrations of 4.44 ± 0.03, 3.90 ± 0.16 and 3.50 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample A had the highest overall zinc concentration with time, while sample C had the lowest zinc concentration as shown in Figure 3.

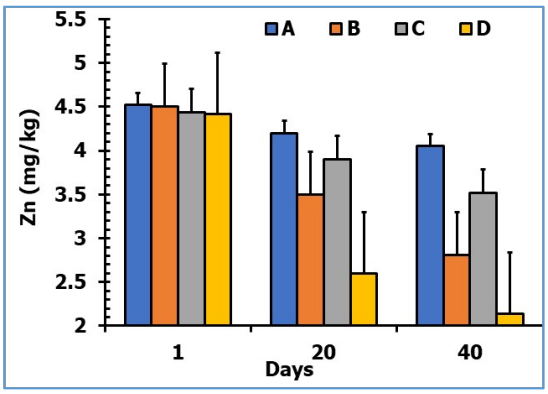

Figure 3: Changes in zinc concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

The copper concentration of the different treatments decreased with time as shown in Figure 4. The copper concentration for sample A was 1.39 ± 0.01, 1.4 ± 0.02 and 0.95 ± 0.01 mg/kg on days 1, 20 and 40 respectively. Sample B had 1.34 ± 0.03, 0.08 ± 0.00 and 0.75 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample C had 1.32 ± 0.01, 0.92 ± 0.01 and 0.62 ± 0.03 mg/kg on days 1, 20 and 40 respectively, while sample D had 1.30 ± 0.01, 1.00 ± 0.00 and 0.81 ± 0.01 mg/kg on days 1, 20 and 40 respectively.

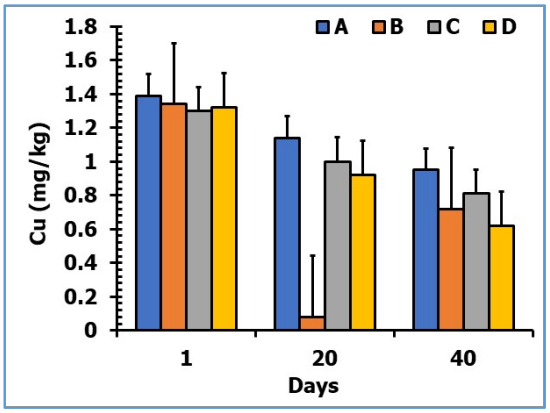

Figure 4: Changes in copper concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

The lowest copper concentration was observed in sample C on day 40, while sample A had the highest concentration of copper on day 1.

The iron concentration was monitored with time in the different treatments (Figure 5).

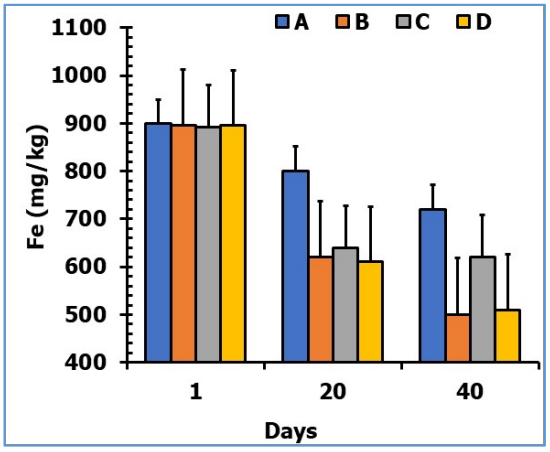

Figure 5: Changes in iron concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

Sample A had an iron concentration of 898.77 ± 0.02 mg/kg on day 1, 800.25 ± 0.03 mg/kg on day 20 and 720.77 ± 0.01 mg/kg on day 40. Sample B had 896.20 ± 0.01 mg/kg on day 1, 620.73 ± 0.02 mg/kg on day 20 and 500.56 ± 0.02 mg/kg on day 40. Sample C had 895.23 ± 0.02, 610.24 ± 0.03 and 510.18 ± 0.02 mg/kg on days 1, 20 and 40 respectively. For sample D, iron concentration was 892.40 ± 0.02, 640.03 ± 0.03 and 620.32 ± 0.02 mg/kg on days 1, 20 and 40 respectively. There was an overall reduction in iron concentration with time in the various treatments. Sample A had the highest iron concentration, while sample B had the lowest.

With time, there was an overall decrease in nickel concentration in the different treatments. Sample A had a concentration of 4.55 ± 0.02, 4.02 ± 0.02 and 3.72 ± 0.01 mg/kg on days 1, 20 and 40 respectively. Sample B had 4.48 ± 0.02, 4.13 ± 0.02 and 3.54 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample C had 4.50 ± 0.01, 4.11 ± 0.01 and 4.05 ± 0.01 mg/kg on days 1, 20 and 40 respectively, while sample D had nickel concentrations of 4.47 ± 0.02, 4.05 ± 0.02 and 3.92± 0.01 mg/kg on days 1, 20 and 40 respectively. Sample A had the highest nickel concentration, while sample B had the lowest, as shown in Figure 6.

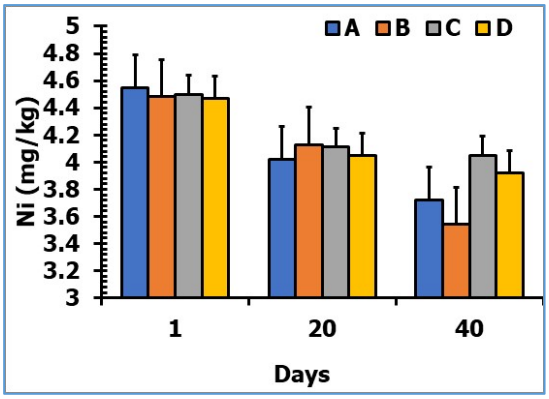

Figure 6: Changes in nickel concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

The chromium concentration of the different treatments was also monitored with time (Figure 7). There was a decrease in chromium concentration with time in all the samples. Sample A had chromium concentrations of 11.13 ± 0.022, 10.18 ± 0.02 and 10.0 ± 0.00 mg/kg on days 1, 20 and 40 respectively. Sample B had 11.1 ± 0.00, 10.56 ± 0.02 and 10.12 ± 0.02 mg/kg on days 1, 20 and 40 respectively. Sample C had 10.99 ± 0.00, 10.2 ± 0.01 and 10.11 ± 0.01 mg/kg on days 1, 20 and 40 respectively, while sample D had a concentration of 11.0 ± 0.00, 10.84 ± 0.02 and 10.48 ± 0.01 mg/kg on days 1, 20 and 40 respectively. Sample D had the highest chromium concentration on day 40, while sample A recorded the lowest on day 40.

Cadmium concentrations in the different treatments decreased with time (Figure 8).

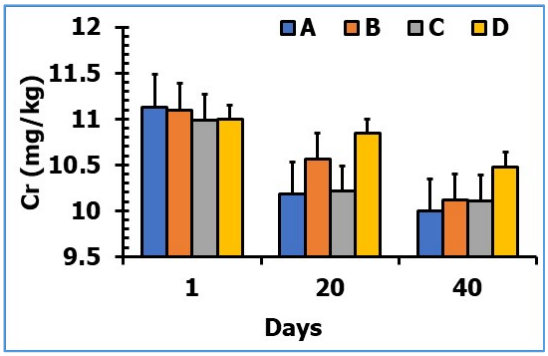

Figure 7: Changes in chromium concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

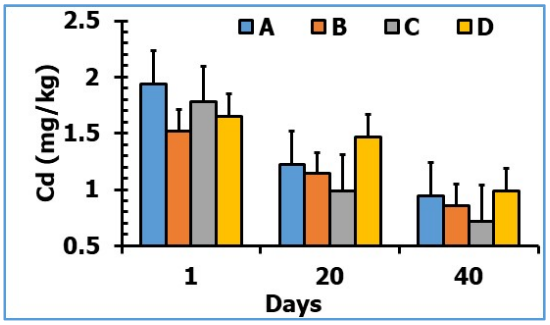

Figure 8: Changes in cadmium concentration during the treatment of crude oil-polluted soil undergoing laboratory-scale bioremediation

Sample A had cadmium concentrations of 1.94 ± 0.01, 1.22 ± 0.02 and 0.94 ± 0.01 mg/kg on days 1, 20 and 40 respectively. Sample B had 1.52 ± 0.01, 1.14 ± 0.01 and 0.86 ± 0.04 mg/kg on days 1, 20 and 40 respectively. Sample C had 1.78 ± 0.01, 0.99 ± 0.01 and 0.72 ± 0.01 mg/kg on days 1, 20 and 40 respectively, while sample D had cadmium concentrations of 1.65 ± 0.02, 1.47 ± 0.02 and 0.99 ± 0.01 mg/kg on days 1, 2, 40 respectively. Sample D had the highest cadmium concentration on day 40, while sample C had the lowest cadmium concentration on day 40.

DISCUSSION

The PAH concentration of the polluted soil samples was high and exceeded the Department of Petroleum Resources (DPR) of Nigeria intervention values for micropollutants and above remediation target values. This high PAH concentration may be due to the degree and duration of pollution. There was a reduction in PAH concentration of the different treatments within the 40 days. Sample A (negative control) experienced the lowest PAH loss of 4.44% and 12.3% at 20 and 40 respectively, because it was unamended. In comparison, sample B (positive control) recorded the highest PAH loss of 43.72% on day 20 and 60.82% on day 40 because of its augmentation using bacterial consortium. This shows the presence of a metabolically active bacterial community that can metabolize hydrocarbons as its sole energy source (Chikere *et al*., 2016; Ezekoye *et al*., 2018). Many researchers have demonstrated bacterial consortiums as containing distinct bacterial groups that can utilise hydrocarbons and plant growth-promoting bacteria because they possess degradative genes and enzymes capable of degrading complex toxic substances like PAHs (Maduwuba, 2024b).

Sample C (test treatment I) was amended using inorganic nutrient supplements, while sample D (test treatment II) was amended using organic nutrient supplements as bio-stimulants to aid the biodegradation process. This explains the reduction in their PAH concentrations. This result agrees with the finding of Uba *et al*. (2019) during the biodegradation of diesel-contaminated soil amended with organic and inorganic nutrients.

The concentration of the heavy metals monitored (Pb, Zn, Cu, Fe, Ni, Cr and Cd) were all below DPR intervention values for micropollutants in soil except for high iron concentration. The high concentration of iron recorded agrees with the studies of Adesina and Adelasoye (2014) and Osu *et al*. (2021), which recorded high iron concentrations in crude oil-polluted soils. This has been attributed to the high degree of crude oil pollution in these soils (Dinakarkumar *et al.,* 2024). During the treatments with NPK and cow dung, there was a poor reduction in the heavy metal concentration in all the treatments, corresponding to the findings of Ogbo and Okhuoya (2011) and Adesina and Adelasoye (2014). This reduction may be a result of increased bioavailability, biotransformation, or solubilization of the heavy metals in the polluted samples and, as such, making it possible and easier for microorganisms to bio-absorb them after releasing them from their bound state (Pande *et al*., 2022). Also, bioaccumulation of these heavy metals in the cell organelles of these

bacteria can be the reason for the reduction in heavy metal concentration, especially in sample B which contains bacterial consortium. The low reduction could be attributed to the high pH, which causes the chemisorptive surface to be more positively charged, thus minimizing the attraction that exists between the metal cations and raising the toxic effect. Low temperatures could also encourage poor bioavailability of heavy metals due to low microbial metabolism and enzymatic activity (Igiri *et al*., 2018; Ubani and Atagana, 2018).

The high iron concentrations in the experimental setup on day 1 may be attributed to the chemical composition of the contaminating crude oil and the possibility that part of the iron components of the pipeline and drilling equipment could have dissolved in the crude oil (Ubani, 2021).

Conclusion: This research has revealed the changes associated with the biodegradation of PAHs and heavy metals in crude oil-polluted soil using different treatment options. The need to develop a metabolically active bacterial consortium capable of degrading PAHs and bio-accumulate heavy metals has also been demonstrated in this study. However, there is an urgent need to harness the indigenous bacterial flora of contaminated sites, enhance such organisms and utilize them in the recovery of impacted sites.

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