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REMEDIATION OF CRUDE OIL POLLUTED SOIL USING A COMBINTION OF MACERATED COBS OF *ZEA MAYS AND PLEUROTUS OSTREATUS*

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ABSTRACT

This study evaluated the bio-absorptive potentials of Pleurotus ostreatus and macerated cobs of Zea mays in the remediation of crude oil polluted soil. Crude oil contaminated soil was collected from B-Dere community located in Gokana L.G.A, Rivers State and analyzed for physicochemical, Total petroleum hydrocarbon (TPH) Polycyclic aromatic hydrocarbons (PAHs) and heavy metal. The crude oil polluted soil sample was divided into five parts of 2,000 g each with the following quantity of macerated cobs of Zea mays, P. ostreatus and triton x-100 added and labeled thus: a) Polluted soil without treatment – cell A (control sample), b) 20ml of triton x-100 - cell B, c) 150 g of macerated cob of Zea mays – cell C, d) 150 g of P. ostreatus – cell D and e) 100 g of macerated cobs of Zea mays and P. ostreatus(i.e. 50 g each) – cell E.Soil samples were collected six (6) weeks after treatment with macerated cobs of Zea mays and P. ostreatus and incubated in sterile plastic bags and transferred to the laboratory for physicochemical, TPH, PAHs and heavy metal analyses. There were significant changes in the mean physico-chemical parameters before remediation (i.e., week 0) and remediation. After 6 weeks of remediation, TPH and PAHs concentration across all the cells were significantly ($p < 0.05$) reduced. The remediation process led to a notable reduction in TPH, PAHs and heavy metal concentrations suggesting its effectiveness in removal of these toxicants. Thus, the combination method is more effective and can enhance bioremediation process as well as solve the problem of waste management and utilization.

INTRODUCTION

Crude oil exploration and application processes causes severe soil contamination owing to inappropriate disposal, leakages of storage tanks, and spills (Wang et al., 2019). Crude oil pollution is of significant environmental concern due to its adverse effects on soil and plant ecosystems. Crude oil-contaminated soils represent a major environmental issue and impose a long-lasting radiation hazard to people's health through the food chains and other pathways (Rahman et al., 2023; Rahman et al., 2023). Crude oil contamination alters soil properties, leading to changes in soil structure, pH, organic matter content, and nutrient availability. Crude oil pollution affects soil physicochemical properties, plant physiology, and ecosystem health. Research by Okafor (2023) found that crude oil pollution significantly decreased soil pH and organic carbon content, while increasing electrical conductivity and hydrocarbon concentrations. These changes affect soil microbial communities and nutrient cycling processes

(Ma et al., 2023). Plants exposed to crude oil pollution experience various physiological and biochemical changes, including reduced growth, photosynthetic efficiency, and nutrient uptake. A study by Li et al. (2021) demonstrated that crude oil exposure led to oxidative stress in plants, characterized by increased levels of reactive oxygen species and lipid peroxidation. Furthermore, crude oil contamination inhibits root development and alters plant hormone balance, affecting overall plant health and productivity (Fatima et al., 2018).

Crude oil pollution poses significant environmental challenges due to its complex composition and the release of various toxic components. Understanding the concentrations of heavy metals, total petroleum hydrocarbons (TPH), and polycyclic aromatic hydrocarbons (PAHs) resulting from crude oil contamination is crucial for assessing environmental impact and devising effective remediation strategies. These pollutants can persist in soil, water, and sediments, leading to long-term ecological damage and health hazards for humans and wildlife. Heavy metals such as lead, mercury, cadmium, and arsenic are often found in association with crude oil due to their presence as trace elements in petroleum reservoirs. Hoober et al. (2022) investigated the distribution of heavy metals in a coastal area affected by an oil spill, revealing elevated concentrations of lead and cadmium in sediments near the spill site. TPH represents a broad category of hydrocarbons present in crude oil, ranging from volatile organic compounds (VOCs) to heavy oils and tars. Assessing TPH concentrations in contaminated environments provides insights into the extent of petroleum contamination and the effectiveness of remediation efforts. Liu and Chen (2022) conducted a comprehensive analysis of TPH concentrations in soil samples collected from a former oil refinery site, demonstrating significant contamination extending beyond the refinery boundaries. PAHs are a group of organic compounds formed during the incomplete combustion of organic materials, including crude oil. These compounds are persistent in the environment and are known carcinogens, posing risks to both human and ecological health (Löffler et al., 2023; Ankley et al., 2020). The presence of PAHs in crude oil-contaminated sites indicates the long-term persistence of hydrocarbon pollutants and underscores the importance of monitoring and remediation efforts. Wang et al. (2020) investigated the spatial distribution of PAHs in sediments impacted by an oil spill, revealing elevated concentrations in proximity to the spill source and highlighting the need for targeted remediation strategies.

Due to the high risk to the health of human beings and ecological safety, crude oil-contaminated soils need to be remediated. Soil remediation associated with crude oil components and organic compounds have aroused intensive concern. Various biological, physical, and chemical technologies have been investigated and widely utilized for the remediation of contaminated sites. Remediating crude oil-polluted soil conventionally often involves physical, chemical, and biological methods (Akpokodje et al., 2019; Adamu et al., 2020). Physical methods include excavation and disposal, while chemical methods use surfactants or solvents. Biological methods employ microorganisms to break down pollutants (Badmus et al., 2021). Traditional methods of remediation often involve costly and environmentally damaging techniques.

Phytoremediati on utilizes plants to uptake, translocate, and metabolize contaminants from soil. Recent research has focused on identifying hyper accumulating plant species capable of absorbing crude oil components. Anwar-ul-Haqet al. (2022) investigated the potential of *Brachiariamutica*for the phytoremediation of crude oil-contaminated soil and observed a significant reduction in TPH levels after a 16-week treatment period. Additionally, Bajraktari et al. (2020) demonstrated the ability of *Salixalba* to accumulate hydrocarbons in its tissues,

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indicating its potential for use in phytoremediation strategies. Microbial remediation involves the use of bacteria and fungi to degrade hydrocarbons present in crude oil. Recent studies have demonstrated the effectiveness of microbial consortia in breaking down complex hydrocarbon compounds. For instance, Ogbonna et al. (2020) observed a significant reduction in TPH in contaminated soil treated with a mixed bacterial consortium over a 12-week period. Similarly, Zhang et al. (2022) reported enhanced degradation of PAHs by a fungal consortium isolated from oil-contaminated soil.

Although bioabsorbtive processes utilizing microorganisms and plants to degrade or absorb contaminants offer a more sustainable and eco-friendly solution, selection of suitable microbial strains or plant species remains a serious challenge to its usage. Many fungi including*P. ostreatus* are hyper-accumulators, suggesting that they are able to concentrate toxins in their fruiting bodies for later removal. This happens via bio-sorption on the cellular surface, which means the metals enter the mycelium in a passive way with very little intracellular uptake in marine environment, waste water and on land (Tastan*et al*., 2016; Vaseem, *et al*., 2017). Hence, the present study is aimed at harnessing the bioapsorptive property of P.*ostreatus*and macerated cobs of *Zeamays* in the remediation of crude oil polluted soil.

MATERIALS AND METHODS

Experimental site and samples collection

Crude oil contaminated soil was collected from B-Dere community located in GokanaL.G.A, Rivers State.

a) Soil sample

Soil samples were collected from the contaminated site from the depth of about 0-25cm and 2kg weighed out into cellophane bag measuring 20cm in height and 20 cm in width.

b) Mushroom (*P. ostreatus*) spawn

The fungus *P. ostreatus* used for this study was obtained from the mycology unit of the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba campus. The culture was sub-cultured in malt extract agar to get pure growing culture.

c) Triton x-100.

One hundred milliliters ((100 ml) of Triton x-100 was purchased from Sigma-Aldrich company, Germany through Bristol Scientific Company Limited, Apapa, Lagos State, Nigeria.

Experimental Design for Soil Treatment.

Two thousand (2000 g) of crude oil contaminated soil sample was taken to the laboratory and analyzed before commencement of the work.

The crude oil polluted soil sample was then divided into five equal partsof 2000 g each with the following quantity of macerated cobs of *Zea mays*, *P.ostreatus* and triton x-100 were added and labeled thus: a) Polluted soil without treatment – cell A (control sample), b) 20 ml of triton x-100 - cell B, c) 150 g of macerated cob of *Zea mays* – cell C, d) 150 g of *P.ostreatus*– cell D and e) 100g of macerated cobs of *Zea mays* and *P.ostreatus* (i.e. 50 geach) – cell E. After treatment with macerated cobs of *Zea mays* and *P.ostreatus*and incubated in sterile plastic bags. The samples were transferred immediately to the laboratory for analysis.

Soil Analysis

The following soil chemical properties were analyzed: physico-chemical properties (pH, phosphate, sulfate, nitrate TOC, total nitrogen), PAHs, TPH, total hydrocarbon content, heavy metals (Pb, Cu, Cd, Mn, Ni)

Determination of Soil pH

Soil pH was evaluated as reported previously (Rowell, 1977). Five grams (5 g) of the soil sample was weighed into a clean beaker. 20 ml of distilled water was added to

it and the sample was stirred with an electromagnetic stirrer for 10 min and allowed to stand for 30 min. The mixture was then stirred again for 2 min and the pH meter electrode was rinsed with distilled water and dipped into the sample in the beaker. Thereafter, the values on the pH meter screen were allowed to stabilize before the reading was taken. This was done for the polluted samples at the beginning of the experiment and at the end of the experiment.

Determination of Soil Nitrate

Soil nitrate was determined as previously describe by Greweling and Peech (1965). One gram (1g) of soil sample was extracted with 50 ml of 2.5 % acetic acid. The extract was filtered into a beaker. 1 ml of extract was pipette into a clean test tube with 0.5 ml of Brucine reagent. 2 ml of concentrated sulphuric acid (H_2SO_4) was added to develop a yellowish colour in the presence of NO_3^- ion. The colour produced was detected at 400 nm using water as blank in a spectrophotometer. Standard nitrate (NO_3^-) was prepared by dissolving 0.722g of potassium nitrate in 100 ml distilled water.

CALCULATION

NO_3^- = N mg/l = Absorbent x standard nitrate graph gradient

Determination of Soil Phosphate concentration

Soil Phosphate level was determined by the Bray No.1 Method as described by Olsen and Sommers (1982). One gram (1g) of soil was extracted with 50 ml of 2.5% glacial acetic acid. The extract was filtered into 250 ml capacity conical flask and 8ml of combined reagent (0.42g selenium powder + 14g lithium sulphate was added to 350ml 30% H_2O_2 and 420ml concentrated H_2SO_4) reagent was added. A blank and standard phosphate ion concentration ranging from 0.0001 and 0.0007 was prepared. 8ml of combined reagent was added. The blue colouration developed within 30mins interval was read at 840nm wavelength in athermo-spectrometerThe volume of the extracted sample was also read at the same wavelength. The concentration of phosphate ion in the sample was extrapolated from the standard phosphate graph plotted. The PO^{4-} values obtained were recorded in mg/kg.

Determination of Soil Total Organic Carbon concentration

Soil total organic carbon content was described by the Walkley and Black method (1934) as modified by Nelson and Sommers 1982). 1g of soil samples collected before and after the experiment was weighed into a 150ml conical flask. 5ml of $K_2Cr_2O_7$solution and 7.5ml concentrated sulphuric acid was added into the sample. The solution was heated for about 30min and allowed to cool. Blanks were also set up with only the reagents excluding samples. A magnetic stirrer was used to ensure proper mixing. The digest was titrated with ferrous ammonium sulphate solution. The end point was a colour change from thick blue to green. The volume of ferrous ammonium sulphate used was recorded as titre value. The blanks were titrated and titre value also recorded.

$$TOC(\%) = \frac{(\text{The blank titre value} - \text{sample titre value}) \times 0.195}{\text{Weight of sample}}$$

Determination of Soil Total Nitrogen (Macro-Kjeldahl Method by Black, 1965)

Soil total nitrogen concentration was evaluated by the Macro-Kjeldahlmethod as described previously by Black (1965). 10g of dry soil sample was weighed into a macro-kjeldahl flask containing 20ml of distilled water. The flask was stirred for a few

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minutes and allowed to stand for 30 min. One tablet of mercury tablet, 10g of K_2SO_4 and 30ml of H_2SO_4 was added to the flask. The flask was heated cautiously at low heat on the digestion stand until the water was removed and frothing ceased. The mixture was then boiled for 5 h. The flask was allowed to cool and 100ml of water was slowly added to the flask. The digest was carefully transferred into another clean macro-kjeldahl flask. Distillation commenced when the flask was attached to the distillation apparatus and about 150ml of 10NNaOH was poured into the distillation flask opening the funnel stopcock. 150ml of the distillate was collected and the distillation was stopped. The NH_4-N in the distillate was determined by titrating with 0.01N standard HCl using 25mlburette graduated at 0.1ml intervals. The colour change at the end point is from green to pink. The percentage (%) Nitrogen content in soil was then calculated.

Determination of soil extractable sulfate

Soil extractable sulfate was determined by the method of Ensminger (1954). 5g of soil sample (air dried, passed 2mm sieve) was weighed into a centrifuge tube and 25ml of KH_2PO_4 solution was added. The tube was shaken on a mechanical shaker for 30 min and Whatman No. 42 filter paper was used to filter the suspension. The SO_4-S content in the solution was determined by the turbidity method.

Determination of Total petroleum Hydrocarbon and Polycyclic Aromatic Hydrocarbons

Total petroleum hydrocarbon content was determined in two successive phases as previously described **TNRCCTx Method 1005, 1997)**

Phase 1: Extraction of Total Petroleum Hydrocarbon:

10ml of dichloromethane (i.e. the extraction of solvent) was added to 2g of the sample. The mixture was thoroughly stirred and allowed to settle and later filtered through extraction column containing cotton wool, sodium sulphate silica gel. The clear extract was collected in extraction bottles and concentrated to 2ml after evaporation.

Phase 2: Gas Chromatographic Analysis:

The concentrated extract solution was then was used for gas chromatographic analysis in HP 5890 series 11 GC machine to determine the values of the various fractions of petroleum hydrocarbon present in the sample. Total petroleum hydrocarbon was obtained by summing the values of the separate fractions detected.

Calculation:

$$\frac{\text{Dilution} \times \text{Reading (TPH)} \times \text{Volume (2ml)}}{\text{Weight of sample(2g)}}$$

Determination of Total Hydrocarbon Content (TNRCCTx Method 1005, 1997)

All water layer on the soil sample was air-dried and sieved through a 2mm mesh size sieve before being thoroughly mixed, especially the composited samples. Foreign objects like sticks, leaves and stones were discarded.

10g of the sample was blended with 10g of anhydrous sodium sulphate, the homogenized sample was transferred to an extraction thimble and covered with glass wool. The extraction thimble was allowed to drain freely for the duration of the extraction period.

Extraction

The soxhlet apparatus containing the extraction thimble and sample was set up with the attachment of a 250ml boiling flask containing 90ml of n-haxane. The heating control on the heating mantle was adjusted so that a cycling rate of 20cycles/hour was obtained. Extraction was carried out for a period of 4 h. Afterwards, a clean 250ml boiling flask was oven-dried at 105°C for 2 h,

after which it was cooled in a desiccator at room temperature. With the use of tongs, the boiling flask was removed from the desiccator and weighed in a calibrated weighing balance.

At the end of the 4 h extraction period, the organic extract was filtered through grease-free cotton into the pre-weighing boiling flask with the aid of hand gloves. The flask and cotton wool were then rinsed with n-hexane and added to the 250ml boiling flask.

The boiling flask was connected to the distilling head apparatus and the solvent was distilled by immersing the lower half of the flask in a heating mantle. The temperature of the heating device was adjusted to complete the distillation in less than 30 min. The solvent was disposed of in a glass bottle designated for storing organic waste before appropriate waste disposal. On complete distillation, the distillation head was removed, followed by the immediate removal of the flask from the heating mantle, before the flask was then cooled in a desiccator for 30 min and weighed. The gain in weight of the boiling flask was determined by subtracting the initial weight from the final weight of flask.

Calculation:

The concentration of hexane extractable material (HEM) in the soil sample is calculated as follows:

$$\text{HEM (mg/kg wet weight)} = \frac{\text{Gain in weight of flask (mg)} \times 1000}{\text{Weight of wet solid (g)}}$$

Determination of Heavy metals (US EPA, 1996)

The levels of heavy metal were evaluated as described previously (US EPA, 1996).

Five grams of air-dried, 2mm sieved soil sample was weighed into a 100ml beaker and 2ml of HNO_3 and 6ml of HCl were added into the beaker in the ratio of 1:3. The mixture was digested by heating on a heating mantle to obtain a near-dryness mixture. The digested sample was filtered using distilled water through a filter paper (Whatman No. 42, 150mm in diameter) into a 50ml volumetric flask. Distilled water was added to make up to 50ml mark digested filtrate in the volumetric flask. The digested soil sample was presented to the atomic absorption spectrophotometer and the concentrations of the selected heavy metals were ascertained. The atomic absorption spectrophotometer was calibrated using standard solutions (solutions of known concentration) for each of the selected metals.

Statistical Analysis of Data

All Data for soil analysis were analyzed for statistical differences by one-way ANOVA and LSD post hoc test using SPSS. In all, $p<0.05$ was considered significant. Data are presented as mean$\pm$S.D (standard deviation).

RESULTS

Physico-chemical Parameters (pH value, Phosphate and Sulfate levels)

After 6weeks of remediation, all samples indicated a significant difference ($p< 0.05$) relative to the mean pH, phosphate and sulfate concentrations of the control sample (cell A).

The mean pH, phosphate and sulfate concentrations of the entire samples, before and after 6 weeks of remediation are summarized in Table 1 below.

Table 1: Soil Physico-chemical Parameters

Cell	Treatment	pH	Phosphate (mg/kg)	Sulfate (mg/kg)
A	Crude Oil Polluted Soil	7.48±0.17	1.09±0.43	2.00±0.00
B	Crude Oil Polluted Soil + Triton x-100	5.15±0.05[a]	0.07±0.03[a]	BDL[a]
C	Crude Oil Polluted Soil +Maize Cob	6.04±0.05[a]	0.38±0.15[a]	BDL[a]

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D	Crude Oil Polluted Soil + *P.ostreatus*	5.33±0.15[a]	0.13±0.02[a]	BDL[a]
E	Crude Oil Polluted Soil +P. ostreatus + Maize Cob	6.03±0.85[a]	0.18±0.01[a]	BDL[a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letter (a) are significantly different at $p \leq 0.05$ when compared with the polluted soil sample. BDL implies below detection limit of 0.01µg/kg wt.

Physico-chemical parameters (Nitrate, Total Organic Carbon (TOC) and Total Nitrogenconentrations)

All samples indicated a significant difference ($p<0.05$) relative to the mean concentration of the control sample (cell A) except Cell E which showed no significant difference relative to the mean nitrate concentration of the control sample (cell A).The mean nitrate, TOC and Total Nitrogen concentrations of the entire samples, before and after 6 weeks of remediation are summarized in Table 2 below.

Table 2: Soil Physico-chemical Parameters

Cell	Treatment	Nitrate (mg/kg)	TOC (%)	TotalNitrogen (%)
A	Crude Oil Polluted Soil without treatment	0.31±0.02	3.25±0.06	0.20±0.07
B	Crude Oil Polluted Soil + Triton x-100	0.42±0.02[a]	5.98±0.04[a]	0.54±0.02[a]
C	Crude Oil Polluted Soil + cobs of *Zea mays*	0.80±0.02[a]	5.56±0.13[a]	0.47±0.02[a]
D	Crude Oil Polluted Soil + *P.ostreatus*	0.48±0.02[a]	5.51±0.12[a]	0.69±0.20[a]
E	Crude Oil Polluted Soil +*P. ostreatus* + cobs of *Zea mays*	0.31±0.03	5.73±0.08[a]	0.48±0.18[a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letter (a) are significantly different at ($p< 0.05$) then compared with the polluted soil sample.

Total Petroleum Hydrocarbon (TPH) Concentration

All samples indicate significant difference ($p< 0.05$)when compared with the untreated crude oil impacted soil sample. The mean TPH concentration from the entire samples, before and after 6 weeks of remediation is summarized in Table 3 below.

Table 3: TPHConcentration

Cell	Treatment	TPH(mg/kg)
A	Crude Oil Polluted Soil without treatment	9635.60±90.74
B	Crude Oil Polluted Soil + Triton x-100	2852.01±35.31[a]
C	Crude Oil Polluted Soil +Cobs of *Zea mays*	2921.41±7.11[a]
D	Crude Oil Polluted Soil + *P. ostreatus*	3086.12±90.40[a]
E	Crude Oil Polluted Soil +*P. ostreatus* + Cobs of *Zea mays*	2334.62±456.89[a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letter (a) are significantly different at ($p< 0.05$) when compared with the polluted soil sample.

Polycyclic Aromatic Hydrocarbon (PAH)

All samples indicate a significant difference ($p< 0.05$)relative to the mean PAH concentration of the control sample (Cell A).The mean PAH concentration of the entire samples, after six (6) weeks of remediation is summarized in Table 4 below

Table 4: PAH Concentration of Cells

Cell	Treatment	PAH (mg/kg)
A	Crude Oil Polluted Soil without Treatment	22.20±1.22
B	Crude Oil Polluted Soil + Triton x-100	6.13±0.00[a]

C	Crude Oil Polluted Soil + Cobs of *Zea mays*	6.60±0.00[a]
D	Crude Oil Polluted Soil + *P. ostreatus*	6.86±0.02[a]
E	Crude Oil Polluted Soil +*P. ostreatus* + Cobs of *Zea mays*	6.13±0.21[a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letter (a) are significantly different at ($p< 0.05$)when compared with the polluted soil sample.

Heavy Metals Concentration

Only sample C indicated a significant difference ($p< 0.05$) relative to the mean copper concentration of the control sample (Cell A).The mean heavy metals concentration of the entire samples, before and after 6 weeks of remediation is summarized in Table 5 below.

Table 5: Heavy Metals Concentration of Cells

Cell	Treatment	Lead (mg/kg)	Copper (mg/kg)	Manganese (mg/kg)	Nickel (mg/kg)	Cadmium (mg/kg)
A	Crude Oil Polluted Soil without treatment	BDL	1.19±0.45	2.25±0.01	BDL	0.12±0.00
B	Crude Oil Polluted Soil + Triton x-100	BDL	1.55±0.14	3.53±0.02[a]	BDL	BDL
C	Crude Oil Polluted Soil +*Zea mays*	BDL	1.73±0.02[a]	4.08±0.07[a]	BDL	BDL
D	Crude Oil Polluted Soil + *P. ostreatus*	BDL	1.00±0.02	4.16±0.01[a]	BDL	BDL
E	Crude Oil Polluted Soil +*P. ostreatus* + cobs of *Zea mays*	BDL	1.05±0.01	4.73±0.02[a]	BDL	BDL

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letter (a) are significantly different at ($p< 0.05$) when compared with the polluted soil sample. BDL implies below detection limit of 0.01µg/kg wt.

DISCUSSION

Crude oil spills pose significant environmental threats, impacting soil quality and necessitating effective remediation strategies. This study investigates the potential of macerated cobs of *Zea mays* and *P. ostreatus* in the amendment of crude oil polluted soil. To access the effectiveness of the remediate, physicochemical properties, TPH, PAHs, and heavy metal result of the polluted untreated sample was compared with the results of the polluted and treated samples.

The mean pH of the crude oil-impacted soil before remediation indicated a slightly alkaline condition. After 6weeks of remediation, the pH values varied across different cells (B, C, D, and E). as shown in Table 1. This change in pH may be attributed to microbial activity during the remediation process, affecting the soil's acidity or alkalinity (Wang et al., 2021). Several studies have highlighted the impact of hydrocarbon contamination on soil pH. According to Smith et al. (2021), microbial activities during bioremediation can influence pH levels in contaminated soils, leading to fluctuations in acidity or alkalinity. The phosphate concentration in the crude oil-impacted soil before remediation was higher than the values obtained after remediation.The variation in phosphate levels could be attributed to the activities of remediation agents, such as microorganisms or amendments, influencing the availability of phosphate in the soil (Kirui et al., 2022). The sulphate concentration after 6weeks of remediation in cells B, C, D and E revealed values below detection limit. Sulphate concentration was significantly reduced across cells B, C, D and E. Sulphate reduction is a known process during microbial degradation of hydrocarbons (Horel and Schiewer, 2020).

The changes in nitrate concentrations across different cells after 6 weeks of remediation suggest variations in the effectiveness of the

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remediation process in different cells. The increase in nitrate concentration suggests a potential transformation of nitrogen-containing compounds during the remediation process. This could be attributed to microbial activities involved in the degradation of hydrocarbons, leading to the release of nitrogen compounds. Recent study by Zheng (2023) demonstrated the effectiveness of microbial-assisted remediation techniques in enhancing nitrate levels in contaminated soils, supporting the observed increase in nitrate concentrations during post-remediation.

Similar to nitrate concentration, TOC levels also experienced significant increase during post remediation. The significant increase in TOC levels across all cells indicates the effectiveness of the remediation process in enhancing organic matter content. This might be attributed to the decomposition of crude oil hydrocarbons by microbial communities (Ugochukwu, 2018; Hu et al., 2019). The significant increase in TOC levels across all remediation cells indicates the addition of organic matter, potentially from microbial biomass or organic amendments used during the remediation process. Study by Johnson et al. (2022) highlighted the role of biochar amendments in enhancing TOC levels and promoting soil microbial activity, which aligns with the observed TOC increase in this study.

The observed variations in the physicochemical parameters suggest the effectiveness of the remediation process in altering the soil conditions. These changes may be attributed to the specific remediation techniques employed, such as bioremediation or phytoremediation, which could have influenced nutrient levels and organic carbon content in the soil. Cui et al. (2023) emphasize the importance of understanding microbial communities in soil remediation processes. Microbial activity plays a crucial role in nutrient cycling and organic matter decomposition, influencing the observed changes in nitrate, TOC, and total nitrogen concentrations. Additionally, studyby Jones and Brown (2022) highlights the role of plant-microbes interactions in phytoremediation processes, offering insights into the potential mechanisms behind the variations observed in this study.

The initial TPH concentration in the crude oil-impacted soil sample indicates a substantial contamination level in the soil at the beginning of the study. Similar high TPH concentrations in oil-contaminated soils have been reported in studies by Almutairi et al. (2020) and Kim et al. (2021). After 6weeks of remediation, the mean TPH concentrations in cells B, C, D, and E were significantly reduced. This aligns with findings in the literature, where successful remediation has consistently resulted in a significant decrease in TPH concentrations (Xiao et al., 2023). This reduction indicates the effectiveness of the remediation process in mitigating the impact of crude oil contamination. Similar positive outcomes in TPH reduction through various remediation techniques have been reported by Jebeli et al. (2018) and Wu et al. (2016). The differences in TPH concentrations among the remediated cells may be attributed to the specific remediation techniques employed in each case. For instance, study by Chen et al. (2020) have highlighted the influence of microbial remediation and phytoremediation in achieving TPH reduction. The observed reduction in TPH concentrations suggests the efficacy of the remediation process employed in this study. Various remediation techniques, such as bioremediation, phytoremediation, and chemical treatment, have been documented to effectively reduce TPH concentrations in crude oil-contaminated soils. Bioremediation, which involves the use of microorganisms to degrade pollutants, has been widely studied for its effectiveness in reducing TPH levels in contaminated soils (Smith et al., 2020). Microorganisms such as bacteria and fungi have the ability to metabolize hydrocarbons, leading to their breakdown into less harmful compounds. This may explain the significant reduction in TPH concentrations observed in the treated cells. Furthermore, the use of specific plants

in phytoremediation has also shown promise in reducing TPH levels in contaminated soils (Li et al., 2019). Plants can enhance the degradation of hydrocarbons through their root exudates, promoting the activity of soil microorganisms. The combination of plant-microbe interactions can contribute to the observed remediation efficiency in the study. The remediation of crude oil-impacted soil is a crucial environmental concern due to the presence of PAHs, known for their harmful effects on the ecosystems and human health (Smith and Brown, 2021). In this study, the mean PAHs concentration before remediation indicated substantial contamination of the soil. The remediation process, implemented over 6weeks, targeted the reduction of PAHs concentrations in the soil. After the treatment period, significant improvements were observed. This aligns with the findings of Kaur et al. (2021), which reported similar reductions in PAH concentrations following remediation treatments in contaminated soils. These results are also in line with recent studies that highlight the efficacy of various remediation techniques in mitigating PAH contamination in soil (Guo et al., 2020; Meng et al., 2023).It is important to note that the decrease in PAH concentrations is essential for environmental health. PAHs are persistent and can have adverse effects on soil quality, water sources, and ultimately impact human health through the food chain (Agency for Toxic Substances and Disease Registry, 1995). This is a positive outcome, suggesting that the remediation process was effective in decreasing the levels of PAHs in the soil. Furthermore, the observed significant difference in the mean PAH a concentration between the treated cells (B, C, D, and E) and the control sample (Cell A) strengthens our argument on the efficacy of the remediations model presented in this study. The outcome of this is also in line with the recommendations of environmental scientists advocating for innovative and sustainable approaches to address soil contamination (Brown and White, 2024).

Furthermore, after 6weeks of remediation, significant changes were observed in the heavy metal concentrations, indicating the effectiveness of the remediation process. The remediation process also led to a notable decrease in Pb, Ni, and Cd concentrations, with all values falling below the detection limit. This suggests the successful removal or immobilization of these toxic metals from the soil. Such remediation achievements align with previous studies emphasizing the potential of various remediation techniques, including bioremediation and phytoremediation, in reducing heavy metal contamination in polluted environments (Smith and Brown, 2022; Nedelescu et al., 2018). Among the heavy metals, copper (Cu) concentrations varied across different remediation cells. Notably, only sample C exhibited a significant difference compared to the control sample (Cell A). This variability in Cu concentrations underscores the importance of considering specific remediation strategies and their impacts on individual heavy metals. It is essential to acknowledge that the efficiency of the remediation processes can be influenced by factors such as microbial activity, soil composition, and remediation agents (Li et al., 2022; Neina, 2019). Manganese concentrations increased in all remediation cells compared to the initial levels, with sample E showing the highest concentration. Manganese is an essential nutrient for plants, and its elevated levels in the post-remediation model might be attributed to the introduction of remediation agents or microbial activity.

CONCLUSIONS

The findings of this study showed that the rate of biodegradation depends majorly on soil nutrient availability and that soil treatment using combination of macerated cobs of *Zea mays* and *P.ostreatus* have proven to be a better potential treatment options for the remediation of petroleum hydrocarbons, PAHs and heavy metal contaminated soil. This combination method can enhance bioremediation process as well

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as solve the problem of waste management and utilization. Overall, the study has proven that using macerated cobs of *Zea mays* or combination with *P. ostreatus* can fit in as a possible organic soil treatment option.

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