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Heavy metals in crude oil-polluted soil and the remediation potentials of heat-stable biocatalytic remediation cocktail (HBRC) and its residue

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Abstract
This study investigated heavy metals in crude oil-polluted soil and the remediation potentials of Heat-Stable Biocatalytic Remediation Cocktail (HBRC) called Garbage Enzyme and its residue (HBRCR). The Crude oil-contaminated soil sample was collected from Agbura Community, Yenagoa, Bayelsa State. HBRC was produced from three substances; water, fruit skin (orange, pineapple, watermelon, plantain and banana peels) and brown sugar in a ratio of 10: 3: 1 respectively. They were subjected to fermentation for 90 days. The soil sample was divided in to ten groups. Group 1 served as the control, Group 2 untreated while Groups 3 to 10 were given different treatments for 180 days. Heavy metals (Hg, Cr, Cd, Pb and Ni) concentrations were analyzed using an Atomic Absorption Spectrophotometer (AAS). Significant decreases at $p \leq 0.05$ of Hg, Cr, Cd, Pb and Ni were observed in groups 3 to 10 when compared to groups 1 and 2. Results obtained from the study revealed that there was a decrease in concentrations of various heavy metals. Hg concentrations in Groups 1 to 10 were reduced by 0.00%, 9.97%, 76.36%, 63.61%, 65.61%, 60.93%, 83.17%, 69.50%, 68.41% and 79.35% respectively. Similarly, Cr concentrations in Groups 1 to 10 were reduced by 19.73%, 11.78%, 96.05%, 36.17%, 51.79%, 74.45%, 92.09%, 55.07%, 85.12% and 80.70% respectively. Also, Cd concentrations in Groups 1 to 10 were reduced by 40.00%, 26.67%, 100.00%, 98.02%, 100.00%, 86.36%, 100.00%, 100.00%, 100.00% and 100.00% respectively. In like manner, Ni concentrations in Groups 1 to 10 were reduced by 18.52%, 33.68%, 45.48%, 69.55%, 47.11%, 44.05%, 51.05%, 64.57%, 65.49% and 65.41% respectively while Pb concentrations in Groups 1 to 10 were reduced by 8.09%, 3.32%, 48.05%, 55.74%, 60.77%, 55.32%, 100.00%, 41.25%, 100.00% and 63.54% respectively. The Results from this study showed that HBRC and HBRCR elicited significant removal of heavy metals from crude oil-contaminated soil and they could be used as remediation agents for heavy metals.

Keywords: HBRC, HBRCR, heavy metals, peels, remediation

Introduction
The occurrence of crude oil with heavy metals in the major ecosystem of the Niger Delta region has created issues on environmental and public health to the front burner and the consequences have attracted the attention of researchers to further explore eco-friendly approaches toward remediation and soil restoration (Vidali, 2001) [1]. Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr) and mercury (Hg) are non-essential elements and are significant pollutants due to their high toxicity and solubility in water (Pinto *et al*., 2004; Benavides *et al*., 2005) [2, 3]. They are metals that tend to form stable dissolved complexes with inorganic and organic ligands, which inhibits their sorption and precipitation (Kubier *et al*., 2019) [4]. Furthermore, cadmium and lead can interrupt enzyme activities and inhibit the DNA-mediated transformation in microorganisms; their primary anthropogenic sources in soils are the direct input of waste material from mining, industry, and agricultural application (Kubier *et al*., 2019) [5]. Lead (Pb) is a toxic non-essential heavy metal, that is widely distributed and induces a wide range of negative effects on living organisms at the morphological, physiological and biochemical level since it is highly persistent in water and soil, accumulates in the upper eight inches of the ground and is highly immobile (Pourrut *et al*., 2011; Tangahu *et al*., 2011) [6, 7].
There are numerous remediation options including chemical and physical techniques. These techniques although yield positive results in bioremediation of heavy metals pollution but in

turn lead to secondary pollution, since they are not eco-friendly (Udofia, 2018) [8] and some are capital intensive. This has prompted an investigation into the utilization of bioremediation using organic methods, and one of such methods is the adoption of a heat-stable biocatalytic Cocktail.

Bioremediation can be explained as processes and products that are cost-effective and practical for the reduction of pollutants in soil sources and diminish the threat to the environment and human health (Kirchhoff, 2003; Rao *et al*., 2014) [9, 10]. Its main methods of degradation and detoxification of pollutants are through intracellular accumulation or enzymatic transformation (Singh *et al.,* 2008) [11].

Enzymes are the most efficient bioremediation tools and progress all chemical changes on pollutants. Enzymes' specificity is usually broad enough to act on different molecules with similar structures (Theerachat *et al*., 2012) [12]. Moreover, it is possible to engineer the enzymes for enhancing their stability and efficiency for special conditions or particular substrates (Festa *et al*., 2008) [12]. Omics technologies have a significant role in these developments (Ufarté *et al*., 2015) [13]. Adoption of enzymes in bioremediation could be either individually where the isolated enzyme used is added to the contaminated soil samples or as a whole cell from microbial agents such as bacteria, fungi, or algae (Eibes *et al.*, 2015) [14]. Secondly, it could be through continuous aeration, inoculation, and nutrition are necessary. Besides, environmental conditions should be prepared for microorganisms living, even though there might still be some harmful molecules in the environment that thwarts the activities of microbes (Rayu *et al.,* 2012) [15]. The use of individual enzymes has some advantages in comparison with microbial whole cell including greater specificity, more straightforward handling and storage, standardizable activity, more mobility as a result of smaller size, being active in the presence of high concentrations of toxic compounds, and biodegradability that inhibits persistence and recalcitrance (Rao, 2010; Gianfreda, and Rao, 2004; Scott *et al.*, 2008) [15, 17, 18], which is much more efficient for extracellular enzymes and cofactor-independent enzymes (Sutherland *et al*., 2004; Scott *et al*., 2008) [19, 20]. This study evaluated the remediation of heavy metals pollutants on crude oil-polluted soil by Heat-Stable Biocatalytic Remediation Cocktail and its Residue.

Materials and Methods

The studied area and Sample Collection

Agbura Community was selected in this study due to the recent crude oil spill that occurred in 2021. The city is the last community in the capital city of Bayelsa State from Ogbia local government. The population of the city is 1200. Agbura is a Community is one of the Communities under Yenagoa Local Government Area of Bayelsa state. It rains most in winter and is moderately warm in summer. Its annual precipitation is 217.7 mm, mean annual temperature is 11.8 °C and 46% humidity.

Soil characters of the area were evaluated as sandy loam containing 80% sand, 12% loam, 6% sludge and 2% organic material with pH 6.8. The identification of soil contamination was also possible based on a visual examination of the soil. The crude oil contaminated soil was collected from the soil, which has a characteristic of black coloration due to oil spillage and the soil surface was hardened. The sample was packaged into a sterile polythene bag and was brought to the Ecological Garden, at the University of Port Harcourt for evaluation. The sample was stored at an adequate temperature (2 to 8 C) before experimental work.

Preparation of Soil Samples

Exactly, 2000 g of the selected soil samples (control and the crude oil contaminated soil) was weighed using analytical balance into thirty (30) different experimental pots. 500 ml each of distilled water was measured and added into the first 18 experimental pots (groups 1 to 6) containing the polluted soil and it was mixed vigorously.

Heat-Stable Biocatalytic Remediation Cocktails (HBRC) Preparation

HBRC was produced from three substances; water, fruit's skin (orange, pineapple, watermelon, plantain and banana peels) and brown sugar in a ratio of 10: 3: 1 respectively. They were subjected to fermentation for 90 Days. The fruits skin (orange, pineapple, watermelon, plantain and banana peels) was obtained from Choba market fruits seller's wing. The water used was fetched from the Post Graduate Hostel Block C Borehole. Twelve liters which is equivalent to 12kg of water was measured using measuring cylinder into an empty clean paint rubber bucket, 1.2kg of brown sugar was weighed using analytical Dial Spring Scale, the brown sugar was dissolved in the water to form sugar solution, 3.6kg of the fruits skin; orange, pineapple, watermelon, plantain and banana peels were weighed in a ratio of 1: 1: 1: 1: 1 and poured into the sugar solution, the whole mixture was properly stirred together for proper mixing and it was covered and labeled with the starting date and end of the reaction date (fermentation was allowed for 90 days). The preparation was set-up using three (3) empty clean paint rubber buckets.

Preparation of Heat-Stable Biocatalytic Remediation Cocktails Residue (HBRCR)

After 90 days of the Fermentation, HBRC was filtered to separate the HBRC from its residue (fruits peels). The residue was sun-dried to obtain dry peels. The oven was avoided to prevent the denaturation of some active ingredients that might be present in the residue. The dry residue was ground into powder form.

Sample Treatment

The soil samples collected in each of the groups (Groups 1-10) were in triplicate, processed and air dried to remove the moisture and water content simultaneously. They were then dried to constant weight in an oven maintained at 105 ºC. Three grams (1.0 g) of the soil samples from each group were carefully weighed into a clean platinum crucible and ashed at 450-500 ºC then cooled to room temperature in desiccators. The sample was dissolved in 5ml of 20% hydrochloric acid and the solution was carefully transferred into a 100ml volumetric flask. The solution was well rinsed with distilled water and transferred to the flask, made up to the mark with distilled water and shaken to mix well. The resulting sample solution from each group was then taken for the determination of the heavy metal (Pb, Cd, Ni, Cr and Hg) concentrations using an Atomic Absorption Spectrophotometer (AAS) based on the procedures of the Association of Official Analytical Chemists (Williams, 2000) [21].

Experimental Design

Soil samples were collected from Agbura Community that was recently polluted by a crude oil spill. The soil samples were prepared and grouped into ten (10). Groups 3 to 6 were treated with HBRCR while Groups 7 to 10 were treated with HBRC as shown below.

S/No (Group)	Treatment
1	Non-Polluted soil Sample, control
2	Polluted but not treated, untreated control
3	Polluted and treated with GER 20t/hectare monthly
4	Polluted and treated with GER 20t/hectare once off
5	Polluted and treated with GER 40t/hectare monthly
6	Polluted and treated with GER 40t/hectare once off
7	Polluted and treated with GE 730t/hectare (25%) monthly
8	Polluted and treated with GE 730t/hectare (25%) once off
9	Polluted and treated with GE 1460t/hectare (50%) monthly
10	Polluted and treated with GE 1460/hectare (50%) once off

Every 30 days the soil samples were taken to the laboratory and the heavy metal levels in them were analyzed using standard reagents and methods which lasted for 6 months (180 days)

Determination of Heavy Metal Concentrations

Principle

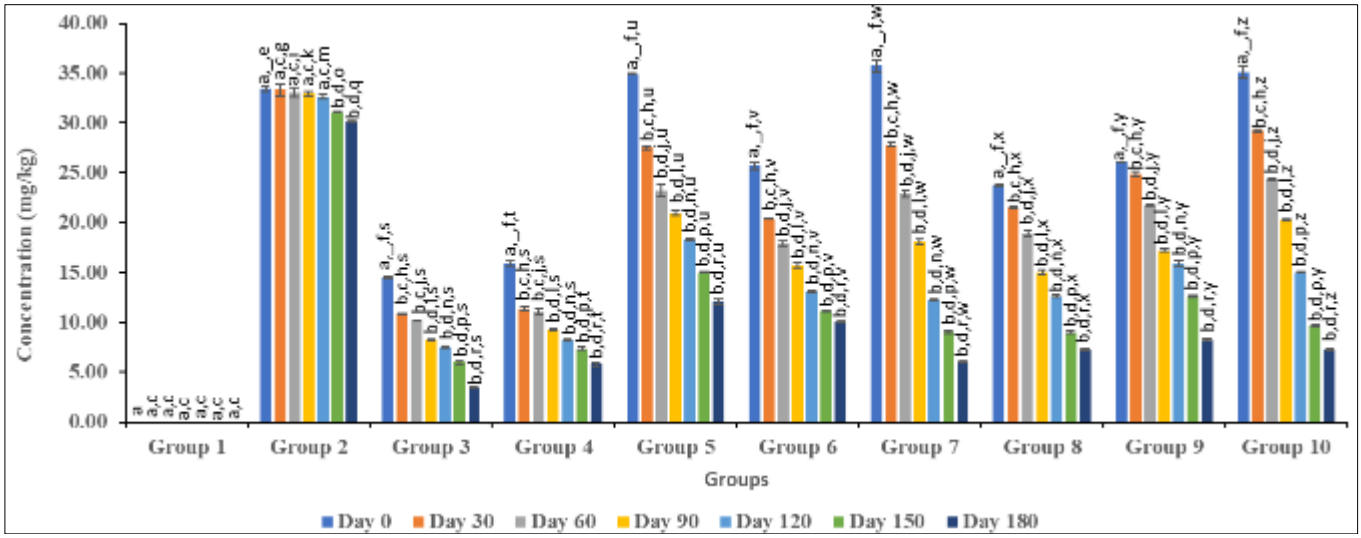
This technique uses the principle of absorption spectrometry to determine the level of an analyte present. The concentration of the analyte to establish or set the relation between the determined absorbance and the analyte concentration which depends on the Beer-Lambert law was determined. One gram of the air-dried and ground soil sample was weighed after sieving using 2mm sieve and transferred into a 250 ml conical flask Perchloric acid ($HClO_4$), nitric acid (HNO_3) and H_2SO_4 were mixed in the ratio 1:2:2 and 20ml of the mixed chemicals was transferred into the conical flask that contained the weighed soil sample. The mixture was heated for about (20 minutes) until total white fumes were observed. The digestion was stopped and cooled. After cooling, 20 ml of distilled water was added and boiled to bring the metals present into the solution, allowed to cool and filtered using Whatmann 42 filter paper into 100 ml volumetric flasks and were filled to 100 ml mark with distilled water. The filtrate was transferred into another 100 ml capacity plastic container for analysis using AAS. For every metal analyzed, the AAS was calibrated or zeroed using metal standards of known concentrations and specific bulbs. Three different concentrations of the standard sample were prepared, digested and aspirated directly into the equipment which forms a straight line to indicate the concentration strength by obeying Beer-Lambert Law.

Results and Discussion

Table 1 below shows the percentage removal of mercury from crude oil-polluted soil treated with different concentrations of HBRC and HBRCR. The mercury (Hg) level in group 4 for 30 days was observed to be the highest in concentration followed by those of groups 3, 5, 7, 6, 10 and 8 while the least was those of group 9. The mercury (Hg) level in group 7 for 60 days was observed to be the highest in concentration followed by those of groups 5, 4, 10, 6, 3 and 8 while the least was 9 (Table 1 and Fig 1). The mercury (Hg) level in group 7 for 90 days was observed to be the highest in concentration followed by those of groups 3, 10, 4, 5, 6, and 8 while the least was those of group 9 (Table 1 and Fig 1). The mercury (Hg) level in group 7 for 120 days was observed to be the highest in concentration followed by those of groups 10, 6, 4, 3, 5, and 8 while the least was those of group 9 (Table 1 and Fig 1). The mercury (Hg) level in group 7 for 150 days was observed to be the highest in concentration followed by those of groups 10, 8, 3, 5, 6 and 4 while the least was those of group 9 (Table 1 and Fig 1). The mercury (Hg) level in group 7 for 180 days was observed to be the highest in concentration followed by those of groups 10, 3, 8, 9, 5, and 4 while the least was those of group 6 (Table 1 and Fig 1). Group 7 treatment yielded the best percentage removal of mercury from crude oil contaminated soil. A more significant removal of Hg from crude oil contaminated soil sample, treated with HBRCR was observed in group 3 followed by groups 5, 4 while the least was group 6 when compared with groups 1 and 2 (Table 1 and Fig. 1). A more significant removal of Hg from crude oil contaminated soil sample, treated with HBRC was observed in group 7 followed by groups 10, 8 while the least was group 9 when compared with group 1 and 2 (Table 1 and Fig. 1). Soil sample treated with HBRC (Groups 7, 10, 8 and 9) yielded the highest percentage Hg removal except for Group 3 (HBRCR), hence HBRC reflected Hg remediation potential. Ugboma *et al.* (2020) [21] on heavy metals from artisanal Crude Oil Refinery impacted soil using *Bacillus flexus* and *Pseudomonas aeruginosa* in Ngie Community reported significant decreases in mercury levels in crude oil-polluted soil treated with *Bacillus flexus* and *Pseudomonas aeruginosa* reported that Mercury was greatly reduced by the activities of *Bacillus and Pseudomonas consortium.*

Table 1: Mercury (Hg) Percentage Removal from Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	4.67	10.12	17.88	24.58	26.54	31.28
Group 2	0.27	1.08	1.37	2.31	6.92	9.97
Group 3	25.24	30.00	43.11	48.16	59.02	76.36
Group 4	28.39	30.62	41.88	48.63	53.74	63.61
Group 5	21.30	33.62	40.04	47.51	57.03	65.61
Group 6	20.56	30.14	38.92	49.13	56.59	60.93
Group 7	22.03	35.91	49.55	65.75	74.78	83.17
Group 8	9.42	20.20	36.71	46.73	62.11	69.50
Group 9	4.59	16.41	33.88	38.92	51.71	68.41
Group 10	16.65	30.51	42.29	57.13	72.53	79.35



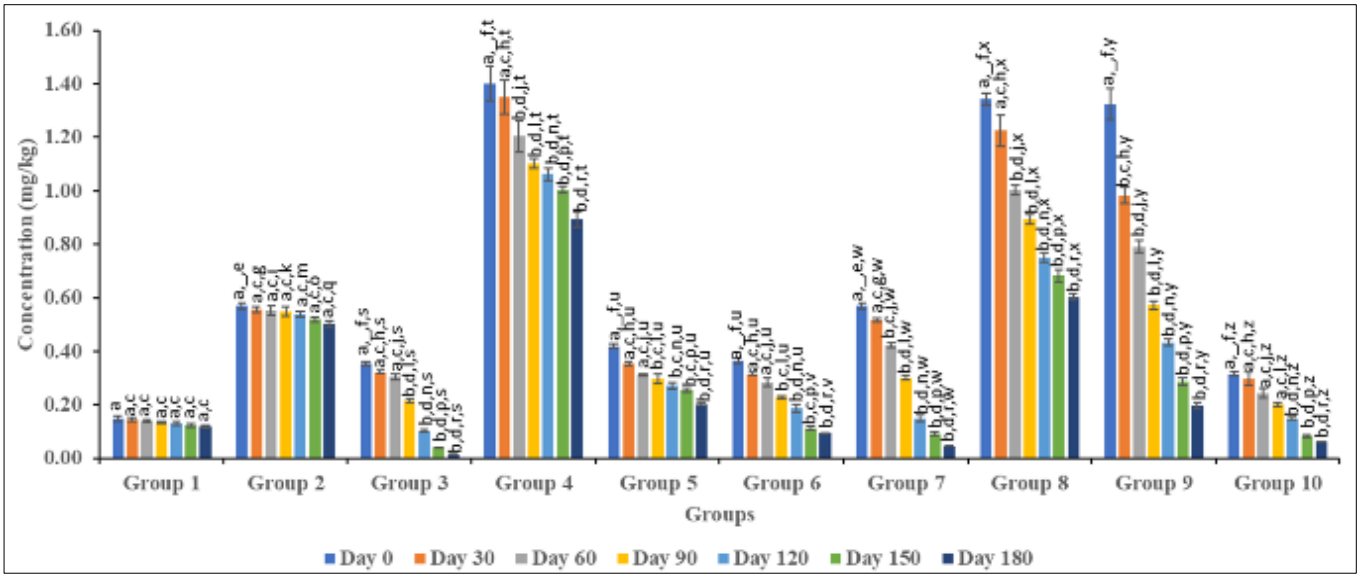
Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at (P< 0.05). Superscript (a, b) compares Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 0 (1st letters) within the group. Superscript (c, d) compares Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 30 (2nd letters) within the group, Superscript (e, f) compares Day 0 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (g, h) compares Day 30 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (i, j) compares Day 60 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (k, l) compares Day 90 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (m, n) compares Day 120 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (o, p) compares Day 150 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2 while Superscript (e, f) compares Day 180 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; (3rd letters) along the column. Superscript (s, t) compares Group 4 to Group 3; Superscript (u, v) compares Group 6 to Group 5; Superscript (w, x) compares Group 8 to Group 7; while Superscript (y, z) compares Group 10 to Group 9 (4th letters) along the column.

Fig 1: Mercury (Hg) Concentrations in Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR

Table 2 below shows the percentage removal of chromium (Cr) from crude oil polluted soil treated with different concentrations of HBRC and HBRCR. The chromium (Cr) level in group 9 for 30 days was observed to be the highest in concentration followed by those of groups 5, 6, 7, 3, 8 and 10 while the least was those of group 4. The chromium (Cr) level in group 9 for 60 days was observed to be the highest in concentration followed by those of groups 7, 5, 8, 10, 6, and 3 while the least was those of group 4 (Table 2 and Fig 2). The chromium (Cr) level in group 9, for 90 days was observed to be the highest in concentration followed by those of groups 7, 3, 6, 10, 8 and 5 while the least was those of group 4 (Table 2 and Fig 2). The chromium (Cr) level in group 7 for 120 days was observed to be the highest in concentration followed by those of groups 3, 9, 10, 6, 8, and 5 while the least was those of group 4 (Table 2 and Fig 2). The chromium (Cr) level in group 3 for 150 days was observed to be the highest in concentration followed by those of groups 7, 9, 10, 6, 8, and 5 while the least was those of group 4 (Table 2 and Fig 2). The chromium (Cr) level in group 3 for 180 days was observed to be the highest in concentration followed by those of groups 7, 9, 10, 6, 8 and 5 while the least was those of group 4 (Table 2 and Fig 2). A more significant removal of Cr from crude oil contaminated soil sample, treated with HBRCR was observed in group 3 followed by groups 6, 5 while the least was group 4 when compared with groups 1 and 2 (Table 2 and Fig. 2). A more significant removal of Cr from crude oil contaminated soil sample, treated with HBRC was observed in group 7 followed by groups 9, 10 while the least was group 8 when compared with groups 1 and 2 (Table 2 and Fig. 2). Soil sample treated with HBRC (Groups 7, 9, 10 and 8) elicited highest percentage Cr removal except for Group 3 (HBRCR), hence HBRC reflected Cr remediation potential which is corroboration with the report of Giwa & Ibitoye, (2017) [22] on the bioremediation of heavy metal in crude oil contaminated soil using isolated Indigenous microorganism cultured with E coli.

Table 2: Chromium (Cr) Percentage Removal from Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	2.04	5.44	8.84	11.56	16.33	19.73
Group 2	2.28	2.99	3.51	5.45	8.79	11.78
Group 3	8.76	14.41	39.55	70.90	88.70	96.05
Group 4	3.50	13.94	21.23	24.02	28.23	36.17
Group 5	15.75	25.54	28.88	35.32	39.38	51.79
Group 6	13.46	21.98	37.36	48.63	69.23	74.45
Group 7	9.31	25.83	46.92	73.81	84.01	92.09
Group 8	8.64	25.26	33.46	43.96	49.18	55.07
Group 9	25.76	40.26	56.80	67.30	78.40	85.12
Group 10	6.33	22.78	36.39	53.16	73.73	80.70



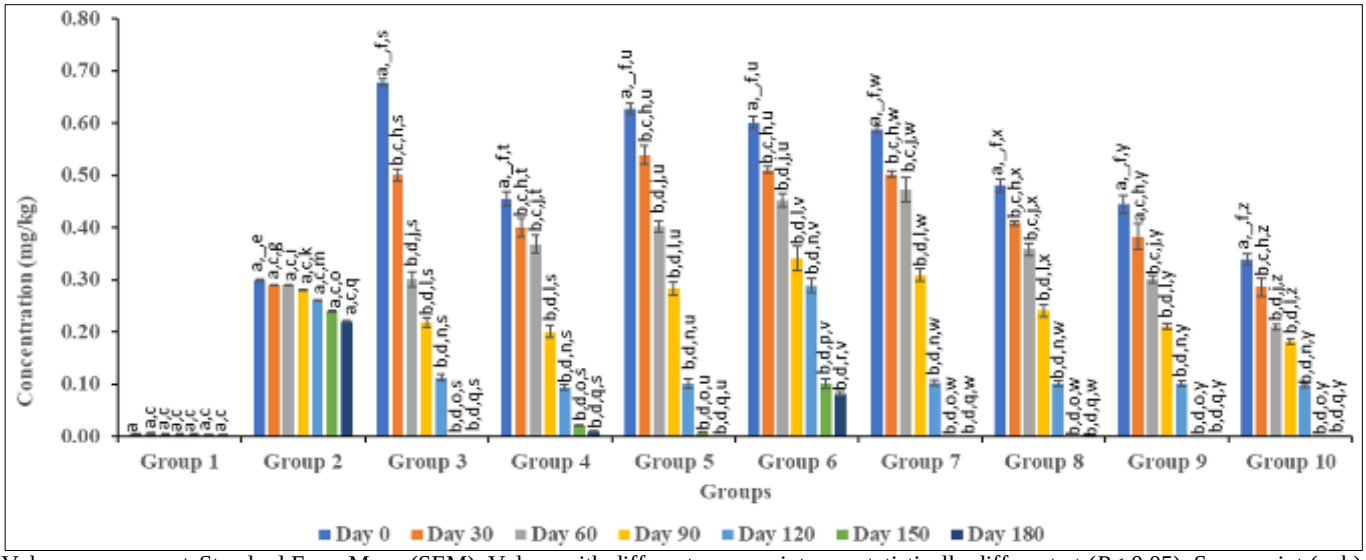
Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at (P< 0.05). Superscript (a, b) compares Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 0 (1st letters) within the group. Superscript (c, d) compares Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 30 (2nd letters) within the group, Superscript (e, f) compares Day 0 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (g, h) compares Day 30 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (i, j) compares Day 60 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (k, l) compares Day 90 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (m, n) compares Day 120 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (o, p) compares Day 150 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2 while Superscript (e, f) compares Day 180 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; (3rd letters) along the column. Superscript (s, t) compares Group 4 to Group 3; Superscript (u,v) compares Group 6 to Group 5; Superscript (w, x) compares Group 8 to Group 7; while Superscript (y, z) compares Group 10 to Group 9 (4th letters) along the column.

Fig 2: Chromium (Cr) Concentrations in Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR

Table 3 below shows the percentage removal of cadmium (Cd) from crude oil-polluted soil treated with different concentrations of HBRC and HBRCR. The cadmium (Cd) level in group 3 for 30 days was observed to be the highest in concentration followed by those of groups 10, 6, 8, 7, 5, and 9 while the least was those of group 4. The cadmium (Cd) level in group 3 for 60 days was observed to be the highest in concentration followed by those of groups 10, 5, 9, 8, 6 and 7 while the least was those of group 4 (Table 3 and Fig 3). The cadmium (Cd) level in group 3 for 90 days was observed to be the highest in concentration followed by those of groups 4, 5, 9, 8, 7 and 10 while the least was those of group 6 (Table 3 and Fig 3). The cadmium (Cd) level in group 5 for 120 days was observed to be the highest in concentration followed by those of groups 3, 7, 4, 8, 9 and 10 while the least was those of group 6 (Table 3 and Fig 3). The cadmium (Cd) levels in groups 3, 7, 8, 9 and 10 for 150 days was observed to be the highest in concentration followed by those of groups 4 and 6 as the least (Table 3 and Fig 3). The cadmium (Cd) level in groups 3, 5, 7, 8, 9, and 10 for 180 days were observed to be highest in concentration followed by those of groups 4 and 6 when compared to groups 1 and 2. A noticeable percentage degradation of Cd from crude oil contaminated soil sample, treated with HBRC and HBRCR was observed in group 3 followed by groups 5 and 4 while the least was group 6 when compared with groups 1 and 2 (Table 3 and Fig. 3). A noticeable percentage degradation of Cd from crude oil contaminated soil sample, treated with HBRC was observed in group 7 followed by groups 8 and 9 while the least was group 10 when compared with groups 1 and 2 (Table 3 and Fig. 3). Soil sample treated with HBRC (Groups 7, 10, 8 and 9) produced the highest percentage of Cd removal except for Groups 3 and 5 (treated HBRCR), which indicated that HBRC possess Cd remediation potential. Fan *et al.* (2022) [23] on the remediation of cadmium and lead in contaminated soils by modified fly ash material, reported decreased Cd and Pb levels on crude oil polluted soil after 80 days of treatment with zeolite (ZO) and modified fly ash (MFA).

Table 3: Cadmium (Cd) Percentage Removal from Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	0.00	20.00	20.00	20.00	40.00	40.00
Group 2	3.33	3.33	6.67	13.33	20.00	26.67
Group 3	26.11	55.60	67.85	83.48	100.00	100.00
Group 4	11.87	19.12	55.82	79.34	95.38	98.02
Group 5	14.17	35.99	54.94	83.97	98.73	100.00
Group 6	14.98	24.79	43.26	51.91	83.19	86.36
Group 7	14.63	19.73	47.45	82.48	100.00	100.00
Group 8	14.97	25.57	49.90	79.00	100.00	100.00
Group 9	13.93	32.43	52.58	77.30	100.00	100.00
Group 10	15.63	38.35	46.31	71.09	100.00	100.00



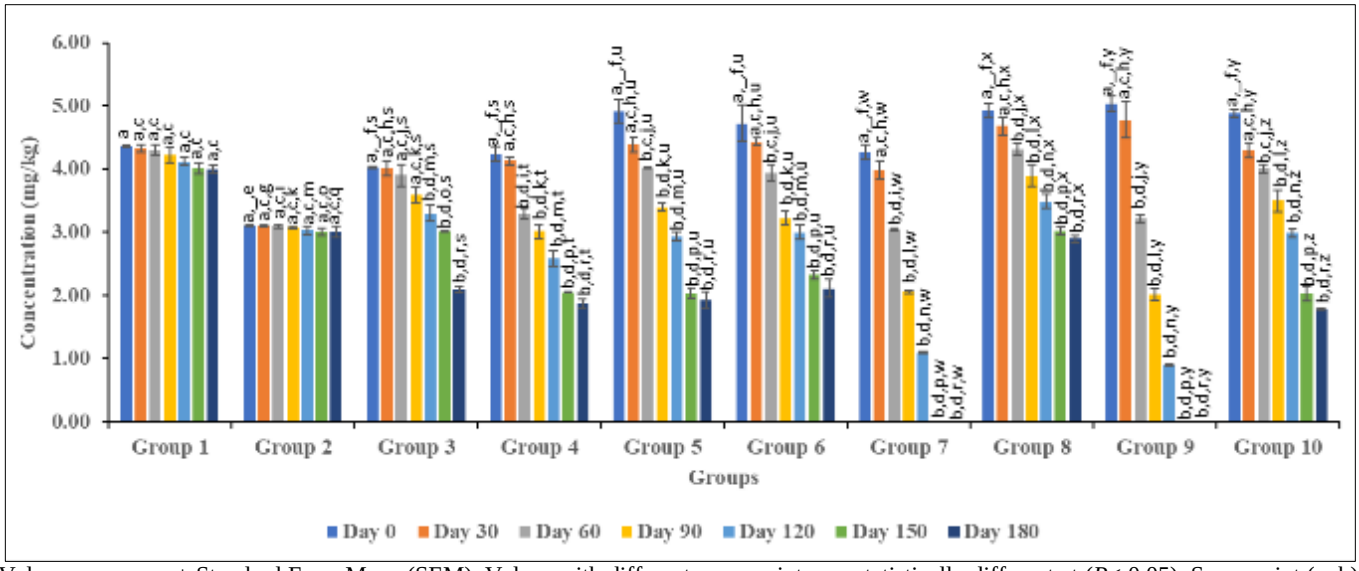
Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at (P< 0.05). Superscript (a, b) compares Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 0 (1st letters) within the group. Superscript (c, d) compares Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 30 (2nd letters) within the group, Superscript (e, f) compares Day 0 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (g, h) compares Day 30 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (i, j) compares Day 60 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (k, l) compares Day 90 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (m, n) compares Day 120 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (o, p) compares Day 150 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2 while Superscript (e, f) compares Day 180 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; (3rd letters) along the column. Superscript (s, t) compares Group 4 to Group 3; Superscript (u,v) compares Group 6 to Group 5; Superscript (w, x) compares Group 8 to Group 7; while Superscript (y, z) compares Group 10 to Group 9 (4th letters) along the column.

Fig 3: Cadmium (Cd) Concentrations in Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR

Table 4 below shows the percentage of lead (Pb) removal from crude oil polluted soil treated with different concentrations of HBRC and HBRCR. The lead (Pb) level in group 10 for 30 days was observed to be the highest in concentration followed by those of groups 5, 7, 6, 9, 8, and 4 while the least was those of group 3. The lead (Pb) level in group 9 for 60 days was observed to be the highest in concentration followed by those of groups 7, 4, 5, 10, 6 and 8 while the least was those of group 3 (Table 4 and Fig 4). The lead (Pb) level in group 9 for 90 days was observed to be the highest in concentration followed by those of groups 7, 6, 5, 4, 10 and 8 while the least was those of group 3 (Table 4 and Fig 4). The lead (Pb) level in group 9 for 120 days was observed to be the highest in concentration followed by those of groups 7, 5, 10, 4, 6 and 8 while the least was those of group 3 (Table 4 and Fig 4). The lead (Pb) level in groups 7 and 9 for 150 days was observed to be the highest in concentration followed by those of groups 5, 10, 4, 6 and 8 while the least was those of group 3 (Table 4 and Fig 4). The lead (Pb) level in groups 9 and 7 for 180 days was observed to be the highest in concentration followed by those of groups 10, 5, 4, 6 and 3 while the least was those of group 8 (Table 4 and Fig 4). A noticeable percentage reduction of Pb from crude oil-contaminated soil sample, treated with HBRCR was observed in group 5 followed by groups 4 and 6 while the least was in group 3 when compared with groups 1 and 2 (Table 4 and Fig. 4). A noticeable percentage degradation of Pb from crude oil-contaminated soil samples, treated with HBRC was observed in group 7 followed by groups 9 and 10 while the least was group 8 when compared with groups 1 and 2 (Table 4 and Fig. 4). Soil sample treated with HBRC (Groups 7, 9, 10 and 8) produced the highest percentage Pb removal except for Groups 5 and 4 (treated HBRCR), which indicated that HBRC possesses Pb remediation potential. Fan *et al.* (2022) [23] in their work on the remediation of cadmium and lead in contaminated soils by modified fly ash material showed that the content of bio-available Pb decreased by 20.6%–28.2% and 29.0%–35.6% after treatment with zeolite (ZO) and modified fly ash (MFA) for 80 days.

Table 4: Lead (Pb) Percentage Removal from Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	0.71	1.19	2.94	5.33	7.84	8.09
Group 2	0.10	0.39	0.97	2.38	3.00	3.32
Group 3	0.47	3.18	10.90	18.32	25.24	48.05
Group 4	2.51	22.20	28.77	38.96	51.58	55.74
Group 5	10.66	18.17	30.93	40.35	58.67	60.77
Group 6	6.19	16.50	31.80	36.61	50.76	55.32
Group 7	6.72	28.75	51.94	74.41	100.00	100.00
Group 8	4.90	12.44	20.91	29.06	38.63	41.25
Group 9	4.97	36.19	60.08	82.09	100.00	100.00
Group 10	12.24	18.07	28.55	38.98	58.67	63.54



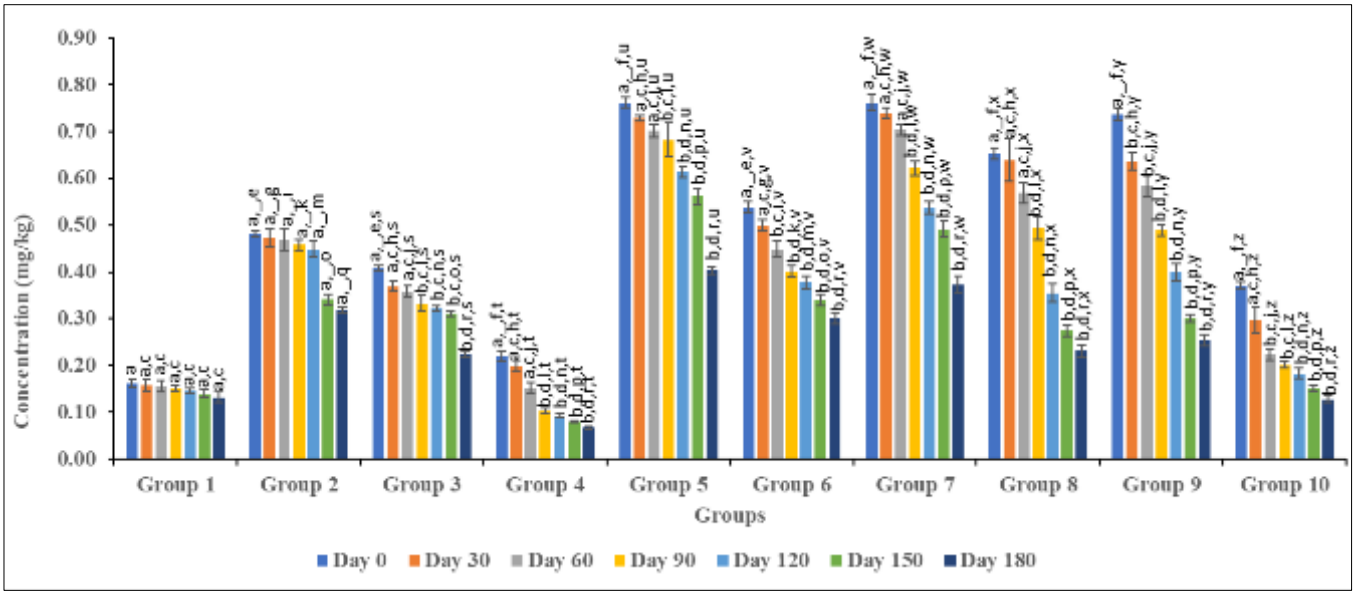
Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at (P< 0.05). Superscript (a, b) compares Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 0 (1st letters) within the group. Superscript (c, d) compares Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 30 (2nd letters) within the group, Superscript (e, f) compares Day 0 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (g, h) compares Day 30 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (i, j) compares Day 60 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (k, l) compares Day 90 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (m, n) compares Day 120 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (o, p) compares Day 150 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2 while Superscript (e, f) compares Day 180 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; (3rd letters) along the column. Superscript (s, t) compares Group 4 to Group 3; Superscript (u,v) compares Group 6 to Group 5; Superscript (w, x) compares Group 8 to Group 7; while Superscript (y, z) compares Group 10 to Group 9 (4th letters) along the column.

Fig 4: Lead (Pb) Concentrations in Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

Table 5 below shows the percentage removal of nickel (Ni) from crude oil polluted soil treated with different concentrations of HBRC and HBRCR. The Ni level in group 10 for 30 days was observed to be the highest in concentration followed by those of groups 9, 4, 3, 6, 5 and 7 while the least was those of group 8. The Ni level in group 10 for 60 was observed to be the highest in concentration followed by those of groups 4, 9, 6, 8, 3 and 5 while the least was those of group 7 (Table 5 and Fig 5). The Ni level in group 4 for 90 days was observed to be the highest in concentration followed by those of group 10, 9, 6, 8, 3 and 7 while the least was those of group 5 (Table 5 and Fig 5). The Ni level in group 4 for 120 days was observed to be the highest in concentration followed by those of groups 10, 9, 8, 6, 7 and 3 while the least was those of group 5 (Table 5 and Fig 5). The Ni level in group 4 for 150 days was observed to be the highest in concentration followed by those of groups 9, 10, 8, 6, 7 and 5 while the least was those of group 3 (Table 5 and Fig 5). The Ni level in group 4 for 180 days was observed to be the highest in concentration followed by those of groups 9, 10, 8, 7, 5 and 3 while the least was those of group 6 (Table 5 and Fig 5). A significant percentage reduction of Ni from crude oil-contaminated soil samples, treated with HBRCR was observed in group 4 followed by groups 5 and 3 while the least was group 6 when compared with groups 1 and 2 (Table 5 and Fig. 5). A significant percentage reduction of Ni from crude oil contaminated soil sample, treated with HBRC was observed in group 9 followed by groups 10 and 8 while the least was in group 7 when compared with groups 1 and 2 (Table 5 and Fig. 5). Soil sample treated with HBRC (Groups 9, 10, 8 and 7) elicited the highest percentage Ni removal except for Group 4 (treated HBRCR), which revealed that HBRC possesses Ni remediation potential. A corresponding significant decrease at $p \leq 0.05$ in Ni concentration with *Eudrilus eugeniae* after 30, 60 and 90 days was reported by Ekperusi and Aigbodion (2015) [24] on bioremediation of petroleum hydrocarbons and heavy metals from crude oil-contaminated soil.

Table 5: Nickel (Ni) Percentage Removal from Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR.

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	2.47	3.70	6.17	10.49	13.58	18.52
Group 2	1.66	2.49	4.78	6.65	29.11	33.68
Group 3	9.78	12.47	18.83	21.52	23.96	45.48
Group 4	10.00	30.91	53.64	57.73	63.64	69.55
Group 5	4.33	7.87	10.50	19.55	26.25	47.11
Group 6	7.25	16.54	25.46	29.74	36.99	44.05
Group 7	3.02	7.74	18.50	29.40	35.43	51.05
Group 8	1.99	12.73	24.39	45.55	57.98	64.57
Group 9	13.59	20.79	33.56	45.79	59.10	65.49
Group 10	19.73	39.73	45.68	50.54	58.92	65.41



Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at (P< 0.05). Superscript (a, b) compares Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 0 (1st letters) within the group. Superscript (c, d) compares Day 60, Day 90, Day 120, Day 150 and Day 180 to Day 30 (2nd letters) within the group, Superscript (e, f) compares Day 0 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (g, h) compares Day 30 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (i, j) compares Day 60 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (k, l) compares Day 90 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (m, n) compares Day 120 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; Superscript (o, p) compares Day 150 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2 while Superscript (e, f) compares Day 180 of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9 and Group 10 to Group 2; (3rd letters) along the column. Superscript (s, t) compares Group 4 to Group 3; Superscript (u, v) compares Group 6 to Group 5; Superscript (w, x) compares Group 8 to Group 7; while Superscript (y, z) compares Group 10 to Group 9 (4th letters) along the column.

Fig 5: Nickel (Ni) Concentrations in Polluted Soil Samples Treated with Different Concentrations of HBRC and HBRCR

Conclusion

This study investigated the heavy metal concentrations in crude oil-polluted soil and the remediation potentials of heat-stable biocatalytic remediation cocktail (HBRC) and its residue. Pollution of soil by heavy metals through crude oil spill is a serious global concern. Hence, environment friendly and cost-effective remediation technology is of great necessity. In this study, soil samples treated with HBRC and HBRCR significantly $p \leq 0.05$ elicited percentage removal of Hg, Cr, Cd, Pb, and Ni levels when compared to groups 1 and 2 from 0 to 180 days. Results obtained from this study revealed that HBRC elicited significant (at $p \leq 0.05$) percentage removal of heavy metals (Cr, Cd, Pb, Ni, Hg) from crude oil-contaminated soil samples when compared to HBRCR. Findings from this study indicated that HBRC and HBRCR elicited significant percentage removal of carcinogenic metals such as (Ni, Pb, Cd and Cr) present in polluted soil, hence could be used as novel means of heavy metal remediation in crude oil impacted soil.

Disclosure statement

The authors declare no conflict of interest.

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Ethical Approval: All authors hereby declared that the principles of laboratory guidelines were followed as well as scientific national laws were applicable. All experiments and procedures were thoroughly examined and approved by the Office of Research Management and Development, Research Ethic Committee (with reference number: UPH/CEREMAD/REC/MM85/020, dated November 24, 2022), University of Port Harcourt, Nigeria.

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