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EFFECT OF HEAT-STABLE BIOCATALYTIC REMEDIATION COCKTAIL (HBRC) ON SELECTED HEAVY METALS PRESENT IN CRUDE OIL-POLLUTED SOIL

***[1]Akpoji, B. S. [1]Essien, E. B. and [1]Nwaichi, E. O.**
[1]Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.
*Corresponding Author: email: bernardsamson2003@yahoo.com
Tel: +2348036886022

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ABSTRACT

This study investigated the effect of a heat-stable biocatalytic remediation cocktail (HBRC) on selected heavy metals present in crude oil-polluted soil. The soil sample was collected from Agbura Community, Yenagoa, Bayelsa State. Heat-stable biocatalytic remediation cocktail (HBRC) also called garbage enzyme (GE) was produced from three substances; water, fruits skin (orange, pineapple, plantain, watermelon and banana peels) and brown sugar in a ratio of 10: 3: 1, and were allowed to ferment for 90 days. The soil sample was divided into six Groups (1 - 6). Groups 1 and 2 served as control; (unpolluted) and (polluted but not treated soil) while Groups 3 to 6 were given different treatments. The samples were prepared and selected heavy metals (Fe, Cu, Zn and Co) concentrations were analyzed using Atomic Absorption Spectrophotometer (AAS). After treatment of the crude oil-polluted soil with the HBRC for 180, there were significant reductions in Fe, Cu, Zn and Co were observed in Groups 3, 4, 5 and 6 when compared to Groups 1 and 2. The highest iron concentration reduction was observed in Group 3 (76.58%). Similarly, the highest Cu concentration reduction was seen in Group 5 (73.55%). Also, the highest Zn concentration reduction was observed in Group 6 (86.30%) while Co highest concentration reduction was seen in Group 5 (86.56%). Hence, the result reveals that HBRC can be used as a remediation cocktail for heavy metals.

Keywords: Garbage Enzyme, Brown Sugar, Fruit skins (peels), Fermentation.

INTRODUCTION

Soil pollution emanating from oil spills has presently attracted global attention (Millioli *et al.*, 2009). The concern stems primarily from health risks posed by direct contact with the polluted soil, vapours from the pollutants, and from secondary pollutants of water supplies within and underlying the soil (Thapa *et al.*, 2012). Accidental spills occur regularly during the exploration, production, refining, transport and storage of petroleum and petroleum products in the Niger Delta region. The release of heavy metal pollutants into the environment or soil whether accidentally or due to human activities is the main cause of water, air and soil pollution. Soil pollution with heavy metal cause extensive damage to the local system since the accumulation of pollutants in animals and plant tissue may cause death or mutation (Nwilo and Badejo, 2001).

Heavy metals are naturally present in the soil. However, geologic and anthropogenic activities accidental spills increase the concentration of these elements to amounts that are harmful to both plants and animals (Akhtar *et al.,* 2021; Shen *et al*., 2002). The group of heavy metals called micronutrients (Fe, Mn, Co, Cu, Zn, Mo), which in excessive quantities are more harmful to plants than animal bodies. The maximum levels of heavy metals in soil and foodstuffs of plant origin should be set at the strictest possible level that is reasonably achievable by good practices of agricultural industry, and taking into account the risks associated with food consumption (Gautam *et al.,* 2022).

Any metals (or semi-metals) species may be considered contaminant if it occurs when it is unwanted or in a concentration or form that causes environmental effects or is detrimental to humans (Sankhla *et al.,* 2016). Some of the metals/metalloids include cobalt, zinc and copper (Singh *et al*., 2011).

In humans, excessive Fe intake can result in Fe overload disorders known as hemochromatosis (Oudit *et al*., 2006). Overdoses of ingested Fe can cause excessive levels of free Fe in the blood (Shander *et al*., 2009). Fe toxicity occurs when the cell contains free Fe which generally occurs when Fe levels exceed the availability of transferrin to bind the Fe (Hershko, 2010).

Copper proteins have diverse roles in biological electron transport and oxygen transportation processes that exploit the easy interconversion of Cu(I) and Cu(II) and is essential in the aerobic respiration of all eukaryotes (Amininia *et al.,* 2013). However, higher concentrations of Cu (100ppm, 200ppm or 500ppm) in the diet of rabbits may favourably influence feed conversion efficiency and growth rates (Kihara, 2013). Chronic Cu toxicity does not normally occur in humans because of the transport system that regulates excretion and absorption (Taylor *et al*., 2020).

The second most abundant trace element in humans after Fe is Zn and it is the only metal that appears in all enzyme classes (Osredkar and Sustar, 2011). Excess Zn is toxic to plants, although, Zn toxicity is far less widespread. The excessive absorption of zinc suppresses Cu and Fe absorption (Kaur and Garg, 2021).

The LD_{50} value for soluble Co salts has been estimated to be between 150mg/kg and 500mg/kg. in the US, the Occupational Safety and Health Administration (OSHA) has designated a PEL in the workplace as a TWA of 0.1mg/m^3 (Kangogo, 2018). However, chronic Co ingestion has caused serious health problems at doses far less than the lethal dose. Furthermore, Co metal is suspected of causing cancer according to International Agency for Research on Cancer (IARC) monographs (Lyon, 2014).

Bioremediation has been globally considered as a method for treating polluted soil which exploits the advantage of the biodegradation, biotransformation and bioaccumulation capabilities of degradative enzymes (Ashoka *et al*., 2002, Zhang *et al*., 2020). Enzymes from fruit waste possess the ability to degrade a wide range of metal pollutants (Akpoji, 2023; Benny *et al*., 2023). The unique capability of enzymes in plants has been exploited to ameliorate crude oil pollution in soils on a laboratory scale. The objective of this study is to evaluate the potential of a heat-stable biocatalytic remediation cocktail (HBRC) in the decontamination of selected heavy metals in crude oil-polluted soil

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 community is located very close to Yenagoa, the capital city of Bayelsa State. Its annual precipitation is 217.7 mm, mean annual temperature is 11.8°C and 46% humidity.

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The identification of soil contamination was also possible based on a visual examination of the soil (crude oil spills, plate 1). The crude oil-polluted soil with a characteristic black colour due to oil spillage was collected and the samples were packaged into a sterile polythene bag. They were transported to the Ecological Garden, University of Port Harcourt for soil identification. The sample was stored at an adequate temperature (22 to 31^{o}C) prior to analysis.

Plate 1: Polluted Site, Agbura Community, Yenagoa, Bayelsa State.

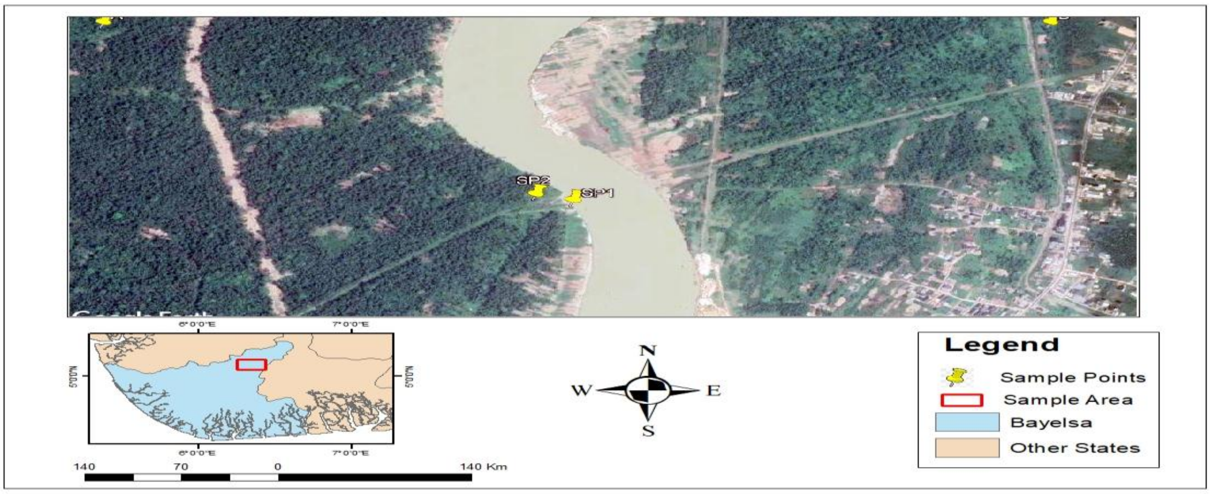

Figure 1: Map of the Study Area, Agbura Community, Yenagoa, Bayelsa State.

Preparation of Soil Samples

Exactly, 2kg of the selected soil samples (control and the crude oil polluted soil) was weighed using an analytical weighing balance into eighteen (18) different experimental pots. Distilled water (500ml) was measured and added into the first six experimental pots containing the unpolluted and polluted soil (Groups 1 and 2) and it was mixed vigorously while Groups 3 to 6 were kept as such.

Preparation of HBRC

HBRC was produced from three materials; water, fruit skin (orange, pineapple, watermelon, plantain and banana peels) and brown sugar in a ratio of 10: 3: 1, were allowed to ferment for 90 Days. The brown sugar was purchased from the Confectionery line (wing), Mile 3 Market, Port Harcourt, Rivers State, Nigeria while the fruit skins (orange, pineapple, plantain, watermelon and banana peels) were obtained from Choba market fruits seller's wing. Twelve liters which is equivalent to 12kg of water was measured using a measuring cylinder into an empty clean paint plastic bucket of 20 liters capacity, 1.2kg of brown sugar was weighed using an analytical dial spring scale, the brown sugar was dissolved in the water to form sugar solution, 3.6kg of the fruit's skins (orange, pineapple, watermelon, plantain and banana peels) were weighed and transferred into the sugar solution. The resulting mixture was properly stirred, covered and labeled with the starting and end of the reaction date. Fermentation was allowed for 90 days. After 90 days of fermentation, HBRC was filtered to separate the HBRC from the residue (fruit peels).

Experimental Design

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

Table 1: Experimental Design

Group	Treatment
1	Non-polluted soil Sample, control
2	Polluted but not treated, untreated control
3	Polluted and treated with HBRC 730t/hectare (25%) monthly
4	Polluted and treated with HBRC 730t/hectare (25%) grab application
5	Polluted and treated with HBRC 1460t/hectare (50%) monthly
6	Polluted and treated with HBRC 1460/hectare (50%) grab application

Every 30 days the soil samples were taken to the laboratory and the heavy metal concentrations were analyzed. The analysis lasted for 6 months (180 days).

Sample Treatment

The soil samples were collected from each of the Groups (1 - 6) in triplicate using amber bottles, processed and air-dried, initially and then to constant weight in an oven maintained at 105ºC. One gram (1.0g) of the soil samples from each group was carefully weighed into clean platinum crucibles, ashed at 450- 500ºC and cooled to room temperature in desiccators. The samples were dissolved in 5ml of 20% hydrochloric acid and the resulting solutions were carefully transferred into 100ml volumetric flasks, 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 heavy metal (Fe, Cu, Zn, and Co) concentrations using an Atomic Absorption Spectrophotometer (AAS) based on the procedures of the Association of Official Analytical Chemists (Williams, 2000).

RESULT AND DISCUSSION

The results obtained showed that the concentration of Fe decreases with increased concentrations of HBRC and with increased time (Figure 2). The Groups with monthly applications of HBRC (Groups 3 and 5) experienced a higher percentage reduction of Fe compared to their counterparts which were given one-off applications; Groups 4 and 6. In groups 3 and 5, Fe percentage reductions were 76.58% and 72.32% while groups 4 and 6 were reduced by 55.05% and 54.96% respectively (Table 2). There were significant decreases ($p \leq 0.05$) when Groups 3 to 6 were compared to Groups 1 and 2. Also, there were significant decreases ($p \leq 0.05$) when the monthly and once applications were compared to each other. Higher percentages reduction of Fe in Groups 3 and 5 might be a result of the nutrient in them that resulted in higher microbial load and consequent degradation of the pollutants when compared

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to Groups 1,2, 4 and 6. Iron (Fe) which is one of the heavy metals is an essential micronutrient plant and play vital roles in almost all living organisms such as photosynthesis, respiration and DNA synthesis (Rout and Sahoo, 2015). Despite the vital roles played by Fe, it becomes carcinogenic at certain concentrations; 305ug/g (kidney cancer), 375ug/g (breast cancer) etc (Mulware, 2013). At a high concentration, iron toxicity occurs and impedes nutrient uptake by plants which lead to the deficiency of nutrients (e.g Zn, K) (Fageria *et al*., 2008) which calls for its remediation. Research has also shown that high concentrations of Fe in the human body can result in liver cirrhosis, diabetes, pancreatic islet damage, liver damage and hypogonadism (Hamada *et al*., 2022). Taiwo *et al*. (2016), remediated contaminated soil using compost and plant technology which successfully removed 29% of iron from the contaminated soil after it was treated with compost during the four weeks of their research. Neculita *et al*. (2011) carried out a comparative study using organic substrate and mushroom for 35 days which resulted in a 21 to 100% reduction of Fe. Similarly, Song *et al*. (2012) carried out similar research using mushroom compost, cow manure, rice straw and sawdust for 174 days and there was 68-92% removal of Fe. Also, Lebrun *et al*., (2019) researched on remediation effect of biochar associated with compost which yielded a significant reduction of Fe. The results indicate that HBRC has the ability to remediate or reduce Fe concentration in the soil.

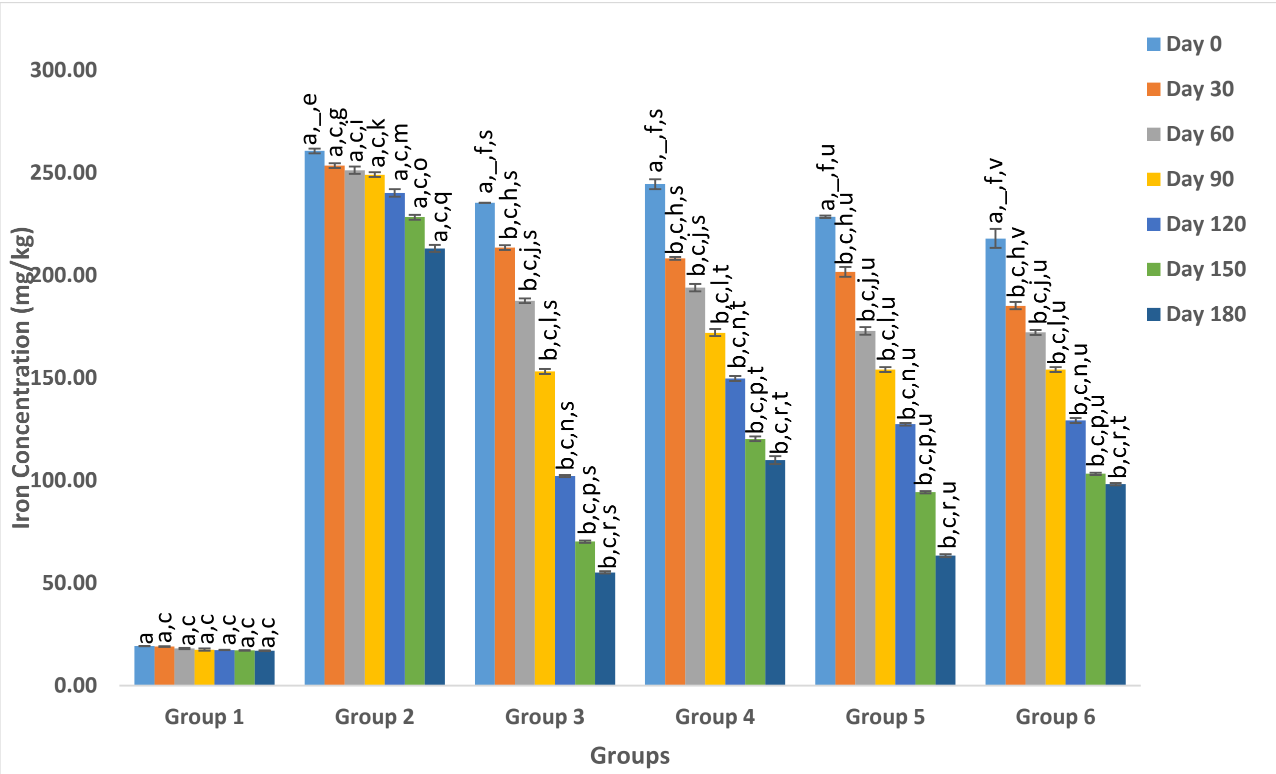

Figure 2: Iron Concentrations in Crude Oil Polluted Soil Samples Treated with Different Concentrations of HBRC.

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.

Table 2: Percentage Reduction of Iron from Crude oil Polluted Soil Treated with Different Concentrations of HBRC

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	1.15	6.25	9.12	9.99	11.09	12.12
Group 2	2.75	3.60	4.44	7.87	12.38	18.26
Group 3	9.28	20.30	34.95	56.60	70.20	76.58
Group 4	14.78	20.62	29.61	38.74	50.81	55.05
Group 5	11.77	24.37	32.62	44.31	58.79	72.32
Group 6	15.02	21.04	29.35	40.74	52.64	54.96

Cu which is one of the chemical elements and heavy metals has roles it plays in both plants and animals (Solayman *et al*., 2016). It is regarded as one of the essential elements (Festa, and Thiele, 2011). Copper is essential for biological roles such as adequate growth, lung elasticity, iron metabolism, neovascularization, neuroendocrine function and cardiovascular integrity (Chen *et al*., 2022; Roy *et al*., 2022). Copper is not among the element classified as carcinogenic by United State *Environmental Protection Agency* (US EPA) (Saleh *et al*., 2019) but excess of copper can result in stunting, inhibition of root growth, leaf discolouration in plants (Martins *et al*., 2016) and loss of chemoreception, inhibited food consumption, cramps, liver damage, diarrhea and abdominal pain in human and some animals (Ling *et al*., 2012). Reduction of excess copper in the soil is therefore necessary. This study showed that the concentration of Cu decreased with an increase in time and concentration of HBRC (Figure 3). The treated groups with monthly application of the HBRC (Groups 3 and 5) yielded higher percentages of removal of Cu when compared to Groups 4 and 6. Copper content in Groups 3 and 5 were reduced by 71.75% and 73.55% while Groups 4 and 6 were reduced by 45.52% and 48.35% respectively (Table 3). There were significant decreases ($p \leq 0.05$) when Groups 3 to 6 were compared to Groups 1 and 2. Also, there were significant decreases ($p \leq 0.05$) when Groups (3 and 4) and (5 and 6) were compared to each other. Higher percentages in reduction of Cu in Groups 3 and 5 might be a result of the nutrients in those groups that resulted in higher microbial population. Adejumo *et al*. (2011) remediated soil-contaminated heavy metal using Mexican sunflower and cassava waste compost and there was the removal of Cu from the soil. Similarly, Meier *et al*. (2017) investigated the effect of chicken-manure-derived biochar (different concentrations: 0%, 5%, and 10%) in the remediation of copper which resulted in the reduction of bioavailability of copper in the soil. Also, Nadaroglu *et al*. (2010) used red mud (activated mud) to remove copper from an aqueous solution.

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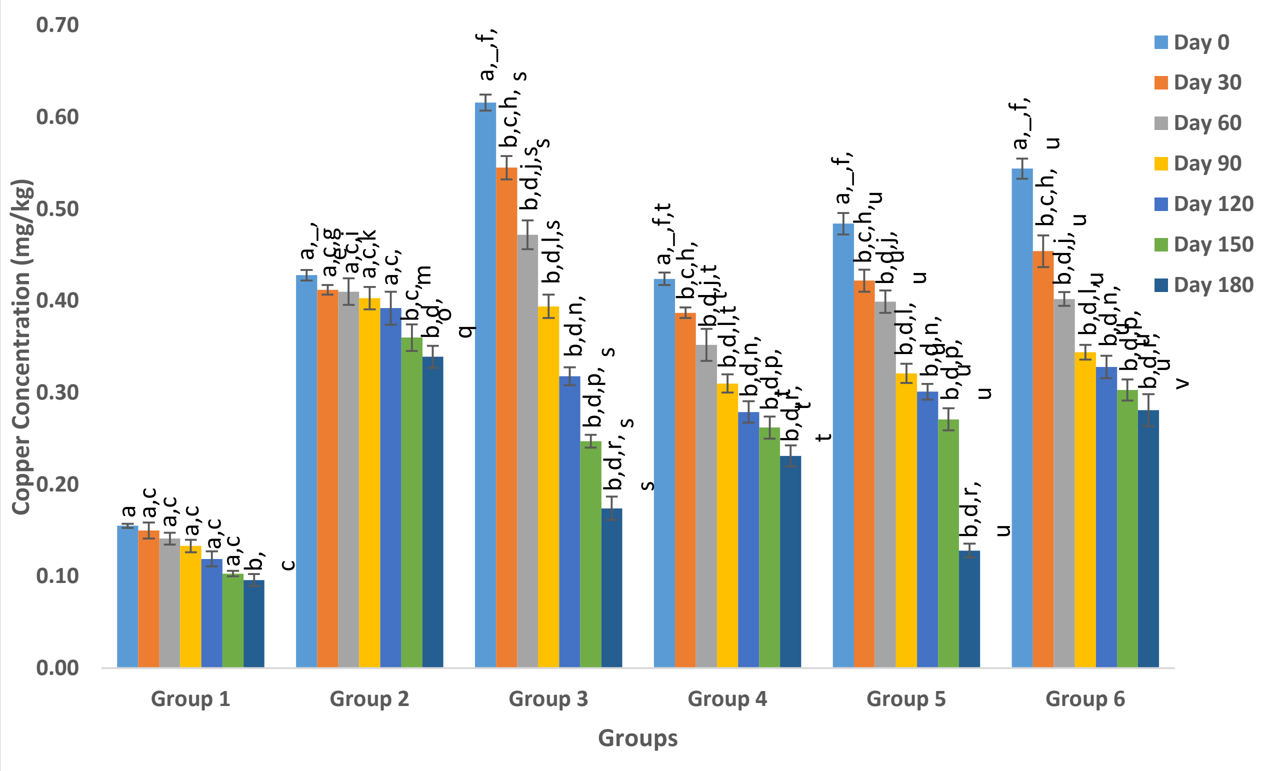

Figure 3: Copper Concentrations in Crude Oil Polluted Soil Samples Treated with Different Concentrations of HBRC.

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.

Table 3: Percentage Reduction of Copper from Crude Oil-Polluted Soil Treated with Different Concentrations of HBRC

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	3.23	9.03	14.19	23.23	33.55	38.06
Group 2	3.74	4.21	5.84	8.41	15.89	20.79
Group 3	11.53	23.38	36.04	48.38	59.90	71.75
Group 4	8.73	16.98	26.89	34.20	38.21	45.52
Group 5	12.81	17.56	33.68	37.81	44.01	73.55
Group 6	16.54	26.10	36.76	39.71	44.30	48.35

The results obtained showed that the concentration of Zn decreases with an increase in time and the concentration of HBRC (Figure 4). The group treated with a monthly application of the HBRC Groups 3 and 5 were reduced by 81.14% and 80.74% while Groups 4 and 6 were reduced by 75.71% and 86.30% respectively (Table 4). There were significant decreases ($p \leq 0.05$) when Groups 3 to 6 were compared to Groups 1 and 2. Also, there were significant decreases ($p \leq 0.05$) when (Groups 3 and 4) and (Groups 5 and 6) were compared to each other. The highest percentage removal of Zn was observed in Group 6. Taiwo *et al*. (2016) remediated contaminated soil using compost and plant technology and the compost removed 29% of Zn within the period of 4 weeks. Farrell and Jones, (2010) discovered that different composts have the potential to remediate zinc and other heavy metal contaminated soil within 64 days experiment which gave significant removal of Zn. Xu *et al*. (2013) produced biochar from dairy manure which was used to remediate heavy metals-contaminated aqueous solution and Zn removal was significant. Soares *et al*. (2015) immobilized Zn using industrial eggshells, potato peels, rice husk and grass clippings which resulted in more than 95% immobilization of Zn. Meng *et al*. (2018) remediated polluted soil using biochar produced from swine manure and rice straw which showed significant removal of Zn, within 150 days. Similarly, Li *et al*., (2019) significantly decontaminated polluted soil using biochar and compost within 120 days. Also, Paradelo *et al*., (2011) reduced the availability of some heavy metals in contaminated soil using municipal solid waste compost which lasted for 90 days and 80% of Zn was removed. The research also revealed that HBRC can be used to reduce Zn concentration in polluted soil. Zinc which is one of the heavy metals is an essential micronutrient that helps in regulating gene expression, enhances protein folding and acts as a cofactor to over 100 enzymes (Mustafa and Komatsu, 2016). Zn enhances wound healing and plays a vital role in carbohydrate breakdown, cell division and growth (Bhowmik *et al*., 2010). It also plays a role in the senses of taste and smell; also needed for proper development and growth of infancy and children (Singh *et al*., 2019; Singh and Mondal, 2019). Zn is not yet classified as carcinogenic by *International Agency for Research on Cancer* (IARC), U.S Department of Health and Human Services (DHHS) and US Environmental Protection Agency (Yadav, 2021). Also, it is not mutagenic and teratogenic. Overdose of Zn can result in headache, indigestion, vomiting and nausea, stomach pain and diarrhea, lower concentration of High-Density-Lipoprotein (HDL), frequent infection and Cu deficiency (Wahlqvist and Wattanapenpaiboon, 2020) high concentration of zinc in the soil could cause general yellowing, wilting and iron deficiency in the plant (McCauley *et al*., 2009).

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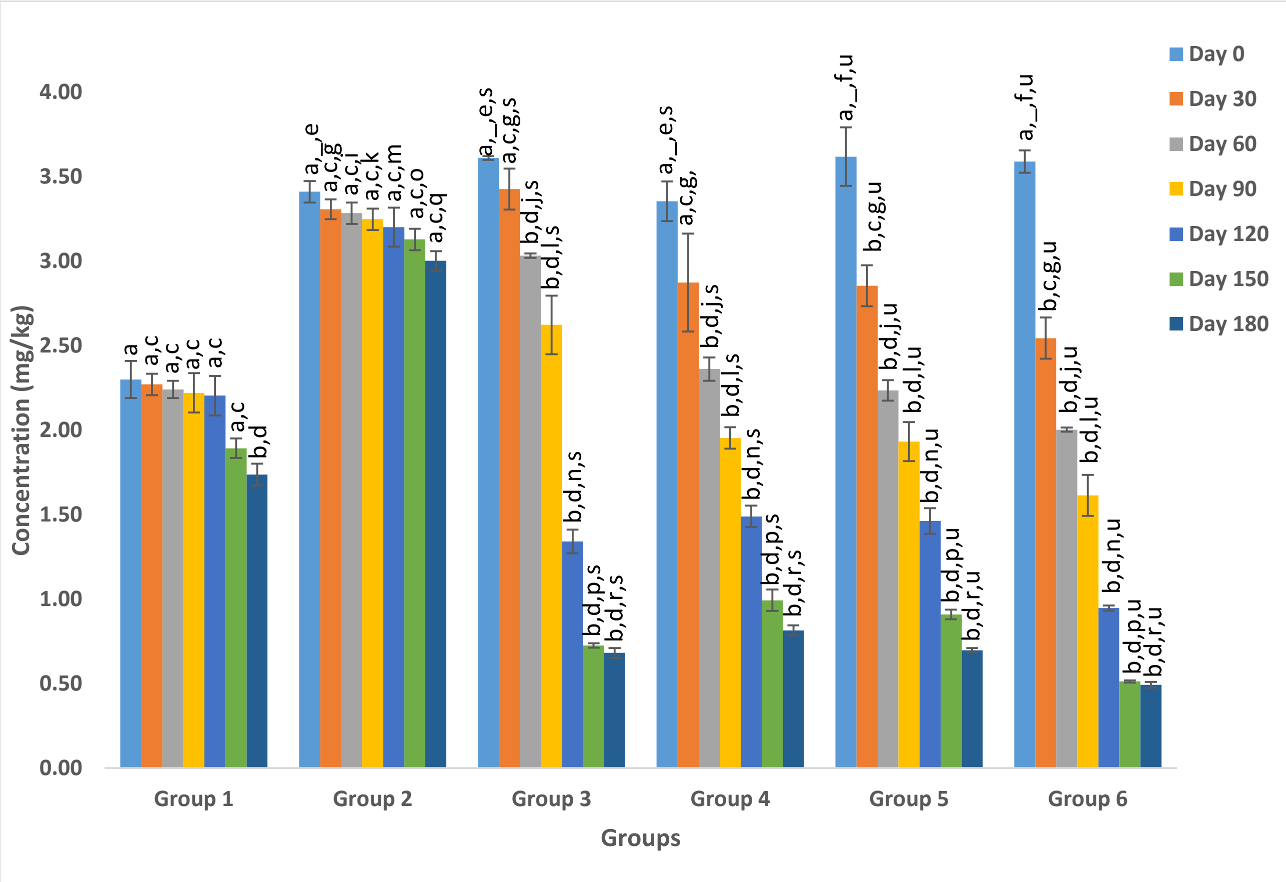

Figure 4: Zinc Concentrations in Crude Oil Polluted Soil Samples Treated with Different Concentrations of HBRC.

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.

Table 4: Percentage Reduction of Zinc from Crude Oil Polluted Soil Treated with Different Concentrations of HBRC

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	1.22	2.52	3.39	4.13	17.66	24.45
Group 2	0.18	0.54	7.37	7.73	8.63	10.07
Group 3	5.10	16.01	27.36	62.86	79.92	81.14
Group 4	14.37	29.60	41.79	55.62	70.40	75.71
Group 5	21.11	38.24	46.62	59.60	74.88	80.74
Group 6	29.11	44.21	55.07	73.62	85.74	86.30

Cobalt which is one of the known heavy metals essential to humans and plants (Nagajyoti *et al*., 2010). Co which can occur in inorganic or organic forms play. It plays vital roles in the human body. Co is a vital component of hydroxocobalamin (Vit. B_{12}) and a major coenzyme of cell mitosis (Bhattacharya *et al*., 2016). Co plays a vital role in the formation of some proteins and amino acids to create myelin sheath in nerve cells (Han *et al*., 2013). Similarly, Co are important parts of enzymes involved in the metabolism of glucose, cholesterol and amino acids and it also play special roles in various catalytic reactions. Aside from the biological roles, Co can also be used in chemical and petroleum industries as a catalyst, to make airbags in automobiles, cemented carbides and corrosion and wear-resistant alloys and also as drying agents for inks, varnishes and paints (Wenzel, 2021). Inorganic forms and excess quantities of Co lead to significant toxicity in water, soil, plants and animals (Pandey and Madhuri, 2014). Excess Co may cause asthma attacks, cough, chest tightness and shortness of breath and may also affect the kidneys, thyroid, heart and liver (Briffa *et al*., 2020). The negative effect of Co calls for the necessity to remediate its excess in soil and water and also to regulate and control plant uptake (Bonilla and Bolanos, 2010). IARC and DHHS have classified Co as carcinogenic but it is yet to be established by US EPA (Dolara, 2014). The concentration of Co decreases with an increase in time and the concentration of HBRC (Figure 5). The treated groups with a monthly application of the HBRC (Groups 3 and 5) yielded higher percentage removal of Co when compared with Groups 4 and 6. Reduction in cobalt in Groups 3 and 5 42.45% and 87.57% while the corresponding values in Groups 4 and 6 was 40.08% and 32.90% respectively (Table 5). There were significant decreases ($p \leq 0.05$) in the level of Co in Groups 3 to 6 when compared to Groups 1 and 2. Also, there were significant decreases ($p \leq 0.05$) when the (Groups 3 and 4) and (Groups 5 and 6) were compared to each other. The highest percentage removal of Co was experienced in Group 5 which might have been as result of the monthly application. Shrestha *et al*. (2019) carried out a comparative study using different composts and coir fibers as remediation agents and the results showed that the compost has the potential to remediate Co. Similarly, Singh and Cameotra, (2013) investigated the efficiency of lipopeptide biosurfactants in the removal of heavy metals and petroleum hydrocarbons and there was a significant reduction in Co (35.4%). Also, Hale *et al*. (2012) treated contaminated soil using cement and lime which removed Co from the contaminated aged soil. The investigation using HBRC reveals that HBRC has the potential to remove Co from crude oil-polluted soil.

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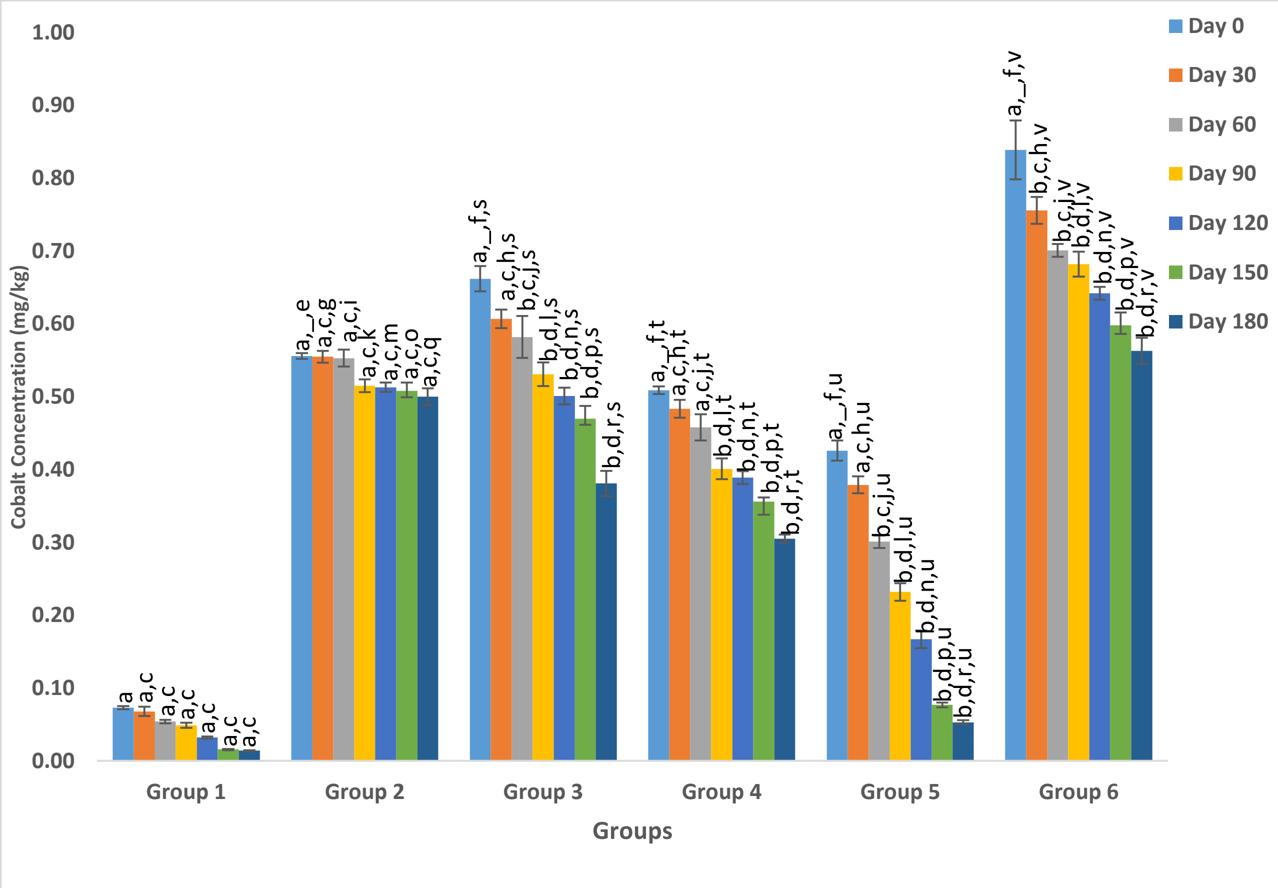

Figure 5: Cobalt Concentrations in Crude Oil Polluted Soil Samples Treated with Different Concentrations of HBRC.

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.

Table 5: Percentage Reduction of Cobalt from Crude Oil Polluted Soil Treated with Different Concentrations of HBRC

	Day 30 (%)	Day 60 (%)	Day 90 (%)	Day 120 (%)	Day 150 (%)	Day 180 (%)
Group 1	6.85	26.03	32.88	56.16	78.08	80.82
Group 2	0.64	1.92	3.85	8.33	10.26	17.31
Group 3	8.31	12.08	19.79	24.32	29.00	42.45
Group 4	5.04	10.02	21.22	23.58	30.06	40.08
Group 5	11.03	29.34	45.54	60.80	81.92	87.56
Group 6	9.89	16.45	18.71	23.48	28.72	32.90

CONCLUSION

The outcome of the research which involved the use of a heat-stable biocatalytic remediation cocktail (HRBC) showed that the cocktail has the potential to remediate heavy metals in crude oil-polluted soil which resulted in a 76.58% reduction in iron concentration, 73.55% reduction of copper concentration, 86.30% reduction of zinc and 87.36% reduction of cobalt within the 180 days of the investigation.

Disclosure statement

No conflict of interest exists between the authors.

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Ethical approval

All authors hereby declare that the principles of laboratory guidelines were followed as well as scientific national laws. 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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