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Biocatalytic Remediation of Heavy Metal on Crude Oil Polluted Soil By Fruit Garbage Enzymes

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ABSTRACT

This research evaluated the biocatalytic remediation of heavy metal on crude oil polluted soil by fruit garbage enzymes (GE). The heavy metals evaluated were Cr, Pb, Ni, Hg, and Cd. The crude oil polluted were divided into 6 groups. Group 1 and 2 served as control and untreated soil respectively. Group 3 was treated with 30% of GE from organic wastes. Group 4 was treated with 50% of GE from plantain and banana wastes. Group 5 was treated with 70% of GE from plantain, watermelon, pineapple and banana wastes. Group 6 was treated with 100% of GE from plantain, watermelon, pineapple, and banana. Group 3, 4, 5, and 6 were treated with GE. The Hg concentration (95.03±0.02%) in group 6 after treatment with 100% of GE were significantly higher than those of group 2 (9.55±0.03%). The Cr concentration in group 6 (68.06±0.02%) after treatment with 100% of GE were higher than those of group 2 (12.04±0.04 %). The Cd concentration in group 6 (81.06±0.04%) after treatment with 100% of GE were higher than those of group 2 (17.33±0.02%). The Pb concentration in group 6 (50.63±0.03%) after treatment with 100% of GE were higher than those of group 2 (3.46±0.03%). The Ni concentration in group 6 (51.82±0.02%) after treatment with 100% of GE were higher than those of group 2 (36.02±0.02%). GE demonstrated biocatalytic remediation of heavy metals on crude oil polluted soil, hence might possibly be an alternative tool for the bioremediation.

***Keywords*:** Crude oil, heavy metals, garbage enzymes, pineapple, plantain, watermelon, banana peels

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1. INTRODUCTION

The increases in the concentration of heavy metals in soil and water in major ecosystem of the Niger Delta area has created environmental and public health issues and the consequences have attracted attention of researchers to traverse eco-friendly approaches toward remediation and soil restoration efforts [1]. Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), and mercury (Hg) are non-essential elements and a significant pollutant due to its high toxicity and solubility in water [2, 3]. They are metals that tend to form stable dissolved complexes with inorganic and organic ligands, which inhibits its sorption and precipitation [4]. Furthermore, cadmium and lead can interrupt enzyme activities and inhibit the DNA-mediated transformation in microorganisms; its primary anthropogenic sources in soils are the direct input of waste material from mining, industry, and agricultural application [4]. 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 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 [5, 6].

There are numerous remediation options including chemical and physical techniques. These techniques although yield positive results in bioremediation of heavy metal pollutants but in turn lead to secondary pollution, due to the fact that they are not eco-friendly [7]. This has prompted investigation into utilization of bioremediation using organic methods, and one of such methods is adoption of heat-stable biocatalysts including Cocktail enzymes.

Garbage enzyme which is a product fermentation of organic waste vegetables, fruits, or its peels along with brown sugar and water which serve as a multipurpose solution for household and agricultural application [8]. Garbage enzymes cannot be produced from any chemical substance. After fermentation, a complex and stable ecological complex that possesses multiple functions emerges, which has elicits three role including: decomposition, combination and biotransformation [9]. The analysis of the active components of garbage enzyme presented that the main active components are various nutrients provided by raw materials of plants and microbes, plant functional chemical components in natural plants, some physiological active substances and various trace elements generated by fermentation, with less toxic and harmful chemicals [10, 11].

Remediation of heavy metals in soil and water through traditional methods can be divided into chemical, physical and biological methods. Chemical additives will result in secondary pollution, and the application of physical repair methods is always restricted for high cost and complex equipment. Bioremediation technology, which is environmental friendly and efficient, is showing a broad application prospect in the field of heavy metal pollution remediation. This has become the principal strategy of in-situ remediation of heavy metal polluted soil in recent years [12]. At present, there are few reports on the application of environmental enzymes in soil heavy metal pollution remediation. In addition, the soils in Agbura Community has been recently heavy metal concentration, posing environmental challenge. Adopting microbial technology can effectively alleviate these problems and conform to the concept of sustainable development.

Remediation of heavy metals in soil and water through traditional methods can be divided into chemical, physical and biological methods [13, 14]. Chemical additives will result in secondary pollution, and the application of physical repair methods is always restricted for high cost and complex equipment. Bioremediation technology, which is environmental friendly and efficient, is showing a broad application prospect in the field of heavy metal pollution remediation. This has become the principal strategy of in-situ remediation of heavy metal polluted soil in recent years [15, 16]. At present, there are few reports on the application of environmental enzymes in soil heavy metal pollution remediation. In addition, the soils in Agbura Community has been recently heavy metal concentration, posing environmental challenge. Adopting microbial technology can effectively alleviate these problems and conform to the concept of sustainable development.

In this study, garbage enzymes were formulated from watermelon, pineapple, ripen banana, and plantain peels from the peels which were used to improve the soil quality. Through the pot experiment, the effects of different garbage enzyme and their percentages on the crude oil contaminated soil and the absorption of heavy metals were studied. The garbage enzyme suitable for the improvement of the soil and the best percentages were selected as the resource of peels of the organic wastes to provide technical guidance and scientific basis for the utilization of resources and the acceleration of soil fertility improvement and pollution remediation in the region.

2. MATERIALS AND METHODS

2. 1. Studied Area and Sample Collection

Agbura community was selected in this study due to the recent crude oil spill that occurred in 2022. The city is located very close to the capital city of Bayelsa State. The population of the city is 1200. Agbura 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 was 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 colors due to oil spillage and the soil surface was hardened. The sample was packaged into a sterile polytene bag and was brought to the Science Laboratory Technology Department, at University of Africa Toru-Orua for evaluation. The sample was stored at adequate temperature before experimental work.

2. 2. Preparation of Soil Samples

Exactly, 200g of the selected soil samples (crude oil contaminated soil, crude oil contaminated sample mixed with cow-dungs at 50:50, and loamy/black soil) was weighed using analytical balance into three different containers. Exactly, 500 ml of distilled water was measured and added into the different container containing the polluted soil and it was mixed vigorously.

2. 3. Garbage Enzyme (GE) Preparation

GE was produced from three organic substances such as ripened pineapple (PA), watermelon (WA), plantain (PL), banana (BN) peels and brown sugar in ratio 10: 3: 1 respectively. The organic substances were subjected to fermentation for the period of 90 Days. The three organic substances (pineapple peels, watermelon and banana peels) were obtained from Swali market Yenagoa. The water used was fetched from the University of Africa undergraduate hostel Borehole. Twelve liters which is equivalent to 12 kg 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 and was dissolved in the water to form sugar solution while 3.6 kg of the pineapple, watermelon and banana peels were weighed and poured into the sugar solution. The mixture were thoroughly stirred together for proper mixing and were covered. The mixtures were then labeled with the starting and end of the reaction dates (fermentation was allowed for 90 days). The preparation was set-up using three (3) empty clean paint rubber buckets.

2. 4. Sample Treatment

The soil samples collected in each of the groups (group 1-10) were 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 (3.0g) of the soil samples from each group was carefully weighed into 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, Hg, Ni, and Cr) concentrations using Atomic Absorption Spectrophotometer (AAS) based on the procedures of the Association of Official Analytical Chemist.

2. 5. Experimental Design

Soil samples were collected from Agbura community, recently polluted by crude oil spill. The soil sample were prepared and grouped into six (6). Group 3 to 6 were treated with were treated with garbage enzymes as shown below.

GROUP 1: Non-polluted soil sample, serving as control

GROUP 2: Polluted and untreated soil sample, serving as untreated soil

GROUP 3: Polluted and treated with GE 730t/hectare (30%) from WA+PA for 30, 60, 90, 120, 150, and 180 days

GROUP 4: Polluted and treated with GE 730t/hectare (50%) from PL+BN for 30, 60, 90, 120, 150, and 180 days

GROUP 5: Polluted and treated with GE140t/hectare (70%) PL+WA+PA+BN for 30, 60, 90, 120, 150, and 180 days

GROUP 6: Polluted and treated with GE1460t/hectare (100%) PL+WA+PA+BN for 30, 60, 90, 120, 150, and 180 days

At the end of the duration for each group, the soil sample carried to the laboratory and the heavy metal levels in them were analyzed using standard reagents and methods

2. 6. Determination of Heavy Metal Concentrations

One gram of each sample was weighed into 50-ml beakers, followed by the addition of 10 ml mixture of analytical grade acids HNO_3 : $HClO_4$ in the ratio 5:1, and left overnight for complete contact of material. Next day, the digestion was performed at a temperature of about 190 ºC for 1.5 h. After cooling, the samples were transferred into 100 ml volumetric flask and solution was made up to a final volume raised up to the mark with distilled water. The metal concentrations were determined by atomic absorption spectrometry using a VARIAN model AA2407 Atomic Absorption Spectrophotometer (AAS). Analysis of each sample was carried out three times to obtain representative results and the data reported in μg g-1 (on a dry matter basis).

2. 7. Statistical analysis

The experiment was designed in completely randomized (CRD) with 6 treatments and three replications. Treatment effects were determined by analysis of variance with the help of statistical package STATISTIX-10 and mean separation was tested by Tukey HSD.

3. RESULTS AND DISCUSSION

The most common problem-causing cationic heavy metals are mercury, cadmium, lead, nickel, copper, zinc and chromium, whereas the most common anionic metal is arsenic. Common organic pollutants at these sites include petroleum, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, herbicides and pesticides [17]. Heavy metal pollution is a significant problem to the ecosystem since these heavy metals particularly cadmium and lead are potentially toxic even at very trace amounts. Cadmium and lead are more perilous because they tend to bioaccumulated [18].

According to Dindar *et al.* [19], a high bioremediation rate was observed when waste water sludge containing heavy metals such as zinc, copper, mercury, chromium, nickel, lead and cadmium was used as a biostimulating agent in the bioremediation of engine oil contaminated soil. In this study, the percentage removal of mercury (Hg) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The Hg levels in the crude oil polluted soil treated with 30% of GE formulated from fermented organic waste from watermelon + ripened pineapple peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 1). The mercury ion levels in the crude oil polluted soil treated with 50% of GE formulated from fermented ripen plantain + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2. Also, the Hg ion concentrations in the crude oil polluted soil treated with 70% of GE formulated from fermented organic waste from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 1).

The levels of Hg observed in the crude oil polluted soil treated with 100% of GE from fermented ripen plantain + watermelon + pineapple + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2. The significant percentage of Hg noticed on crude oil polluted soil sample after treatment with garbage enzyme fermented from organic peel wastes is suggestive that they could be potential option for the removal of increased mercury levels in soil due to crude oil spill. Karic *et al.* [20] on bio-waste valorisation: agricultural wastes as biosorbents for removal of (in) organic pollutants in wastewater treatment and Joglekar *et al.* [21] on process of fruit peel waste biorefinery: a case study of citrus waste biorefinery, its environmental impacts and recommendations, reported similar results.

Table 1. Mean and Standard Deviation of Mercury (Hg) Percentage Removal from Polluted Soil Samples Treated with Different Concentration of Garbage Enzyme (GE) (n=3)

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	4.07 ±0.03[a]	10.25 ±0.02[k]	16.62 ±0.03[k]	24.54 ±0.02[a]	25.45 ±0.04[a]	33.05 ±0.04[a]
Group 2	0.1 ±0.03[b]	1.13 ±0.05[b]	1.32 ±0.02[b]	2.25 ±0.03[b]	6.75 ±0.02[b]	9.55 ±0.03[b]
GE WA+PA (30%)	24.92 ±0.03[c]	29.55 ±0.04[f]	34.94 ±0.03[f]	47.15 ±0.04[f]	55.74 ±0.05[f]	75.06 ±0.02[f]
GE PL+BN (50%)	27.63 ±0.02[c]	31.15 ±0.04[f]	40.26 ±0.02[f]	49.25 ±0.03[f]	56.84 ±0.02[f]	63.23 ±0.03[f]
GE PL+WA+PA+ BN (70%)	22.12 ±0.04[c]	32.75 ±0.04[f]	40.36 ±0.01[f]	48.65 ±0.04[f]	57.25 ±0.04[f]	66.06 ±0.01[f]
GE PL+WA+ PA+BN (100%)	20.34 ±0.02[c]	33.06 ±0.02[f]	39.62 ±0.02[f]	51.44 ±0.03[f]	85.93 ±0.02[f]	95.03 ±0.02[f]

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in means ± Standard Error of Mean (SEM). Values with superscript b are statistically different from group 1 at ($P < 0.05$) down the treatments. Values with superscript c are statistically different from group 1 and 2 at ($P < 0.05$) down the treatments. Values with superscript f are statistically different from group 1 and 2 at ($P < 0.05$) down and across the treatments.

Table 2 shows the percentage removal of chromium (Cr) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The chromium ion levels in the crude oil polluted soil treated with 30% of GE formulated from fermented organic waste from watermelon + ripened pineapple peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 2). Also, the chromium ion concentrations present in the crude oil polluted soil treated with 50% of GE formulated from fermented ripen plantain + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 2).

Again, the levels of chromium ions in the crude oil polluted soil treated with 70% of garbage enzymes (GE) formulated from fermented organic waste from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 2). The concentrations of chromium ion (Cr) in the crude oil polluted soil treated with 100% of GE formulated from fermented ripen plantain + watermelon + pineapple + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 2).

The percentage garbage enzyme removal of Cr in the crude oil polluted soil sample treated with 100% ripen plantain + watermelon + pineapple + banana peel wastes for 30, 60, 90, 120, 150, and 180 days were significantly higher than those treated with 30% of fermented organic peel wastes from watermelon + ripened pineapple peel wastes, 50% of GE formulated from fermented ripen plantain + banana peel wastes, and 70% of fermented organic peel wastes from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days (Table 2). The significant percentage garbage enzyme removal of Cr observed on crude oil polluted soil sample after treatment with the organic peel wastes at 30, 50, 70, and 100% of GE formulated from organic peel wastes, for 30, 60, 90, 120, 150, and 180 days are expressive of the decontamination potential of garbage enzymes from fermented watermelon, ripen pineapple, plantain, and banana peel wastes against heavy metal polluted soil, hence may possibly be a choice for the bioremediation of high Cr levels in soil arising from crude oil pollution.

The percentage garbage enzyme removal of Cr from fermented organic peel wastes perceived in this study is similar to the report of Rafael *et al.* [22] on the gentle remediation options for soil with mixed chromium (VI) and lindane pollution: biostimulation, bioaugmentation, phytoremediation and vermiremediation, which indicated soil health recovery from toxicity due to high Cr (VI) concentrations.

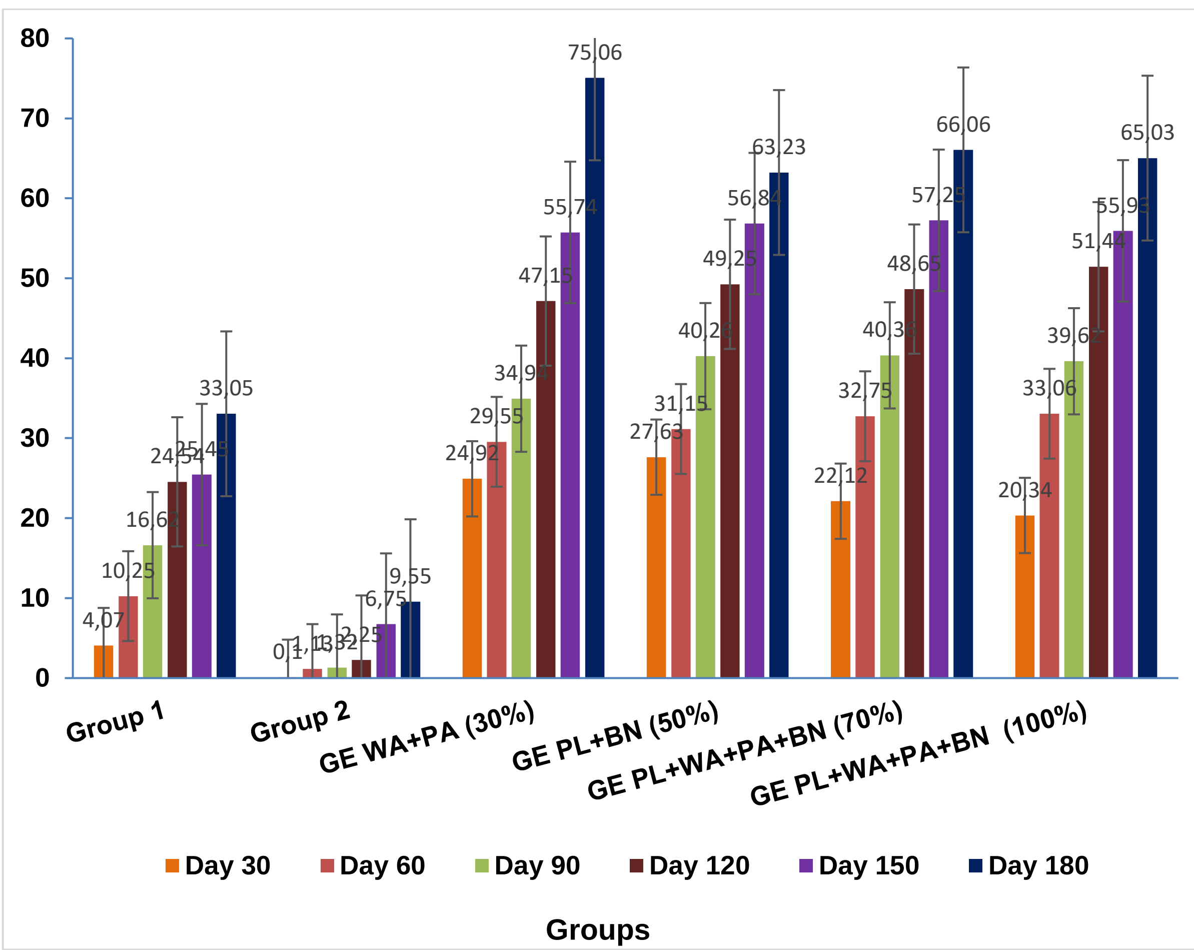

Figure 1. Percentage removal of Mercury (Hg) in polluted soil samples treated with garbage enzyme (GE).

Table 2. Mean and Standard Deviation of Chromium (Cr) Percentage Removal from Polluted Soil Samples Treated with Different Concentration of Garbage Enzyme (GE) (n=3)

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	2.62 ±0.03[a]	5.75 ±0.04[a]	8.64 ±0.03[a]	15.84 ±0.02[a]	19.45 ±0.03[a]	23.04 ±0.04[a]
Group 2	2.37 ±0.03[b]	2.75 ±0.04[b]	3.57 ±0.03[b]	5.44 ±0.03[b]	8.77 ±0.02[b]	12.04 ±0.04[b]
GE WA+PA (30%)	8.76 ±0.03[c]	14.54 ±0.04[f]	39.44 ±0.04[f]	73.15 ±0.04[f]	88.26 ±0.04[f]	94.84 ±0.04[f]
GE PL+BN (50%)	3.56 ±0.02[c]	13.86 ±0.03[f]	21.66 ±0.02[f]	24.17 ±0.02[f]	28.28 ±0.02[f]	36.17 ±0.01[f]
GE PL+WA+PA+BN (70%)	16.25 ±0.02[c]	26.15 ±0.03[f]	37.93 ±0.03[f]	41.23 ±0.03[f]	46.02 ±0.02[f]	55.15 ±0.05[f]
GE PL+WA+PA+BN (100%)	11.94 ±0.03[c]	22.53 ±0.03[f]	36.74 ±0.03[f]	48.15 ±.0.02[f]	52.93 ±0.03[f]	68.06 ±0.02[f]

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in means ± Standard Error of Mean (SEM). Values with superscript b are statistically different from group 1 at ($P < 0.05$) down the treatments. Values with superscript f are statistically different from group 1 and 2 at ($P < 0.05$) down and across the treatments.

Table 3 shows the percentage removal of cadmium (Cd) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The cadmium ion levels in the crude oil polluted soil treated with 30% of GE fermented organic waste from watermelon + ripened pineapple peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 3). Also, the cadmium ion concentrations present in the crude oil polluted soil treated with 50% of GE fermented ripen plantain + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 3). Again, the levels of cadmium ion in the crude oil polluted soil treated with 70% of garbage enzymes (GE) formulated from fermented organic waste from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 3). The concentrations of cadmium ion (Cd) in the crude oil polluted soil treated with 100% of GE fermented from ripen plantain + watermelon + pineapple + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 3).

The percentage GE removal of Cd in the crude oil polluted soil sample treated with 30% GE from fermented watermelon and pineapple peel wastes for 30, 60, 90, 120, 150, and 180 days were significantly higher than those treated with 50% of GE from fermented watermelon + ripened pineapple peel wastes, and 100% GE from ripen plantain, banana, watermelon, and pineapple peel wastes (Table 3). However, the percentage GE removal of Cd in the crude oil polluted soil sample treated with 70% GE fermented ripen plantain, banana, watermelon, and pineapple peel wastes for 30, 60, 90, 120, 150, and 180 days were significantly higher than those treated with 100% of GE from fermented watermelon + ripened pineapple peel wastes, and 100% GE from ripen plantain, banana, watermelon, and pineapple peel wastes (Table 3).

The significant percentage removal of Cd observed on crude oil polluted soil sample after treatment with GE from fermented organic peel wastes at 30, 50, 70, and 100% for 30, 60, 90, 120, 150, and 180 days are evocasive of the decontamination potential of GE from fermented watermelon, pineapple, ripen plantain, and banana peel wastes against soil toxicity due to high Cd levels, hence might perhaps be a recourse for bioremediation of high Cd levels in soil arising from crude oil contamination. The percentage GE removal of Cd levels observed in this study is similar with the report of Bernard *et al*. [23] on toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater.

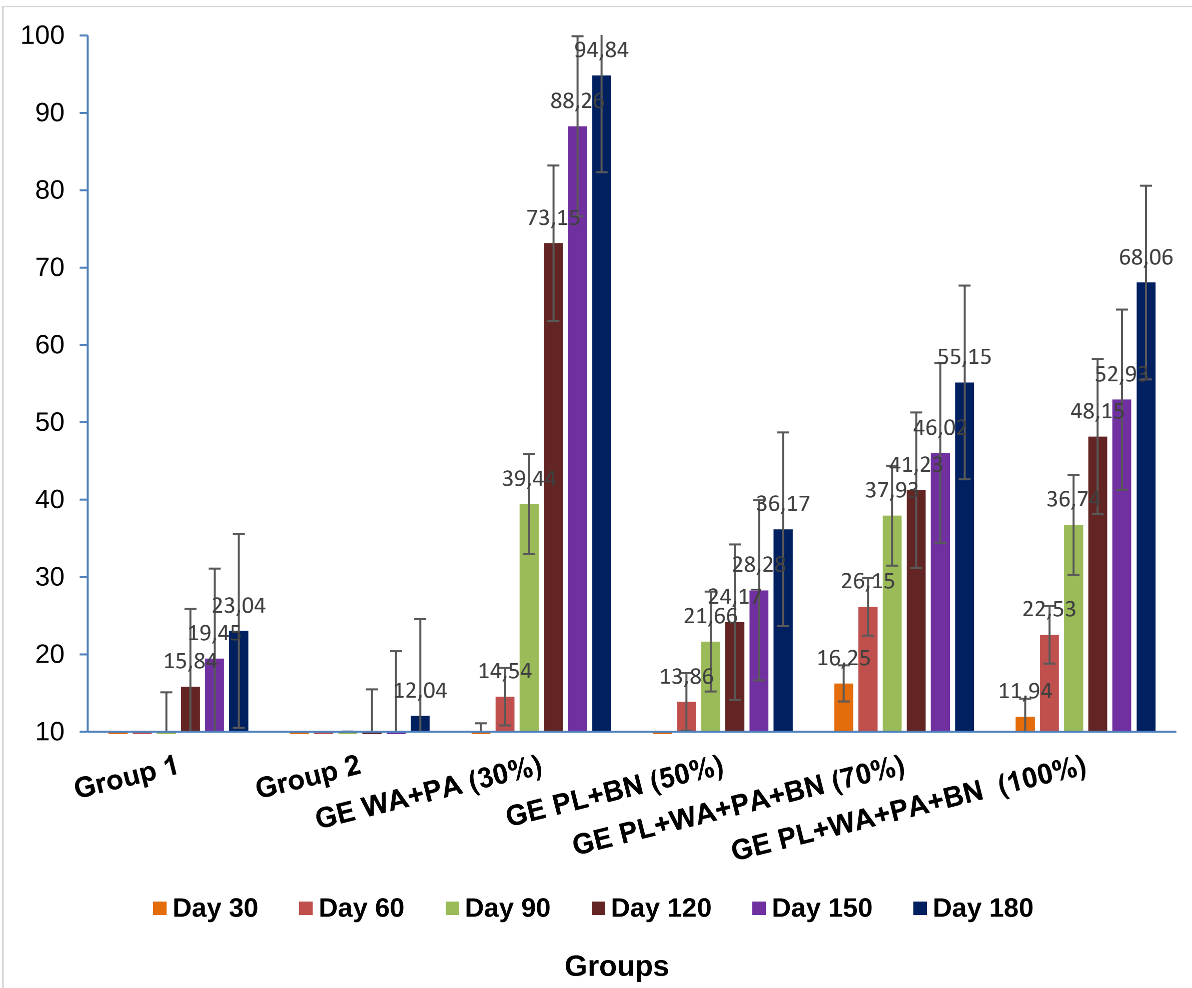

Figure 2. Percentage removal of Chromium (Cr) in polluted soil samples treated with garbage enzyme (GE).

Table 4 shows the percentage removal of lead (Pb) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The Pb levels in the crude oil polluted soil treated with 30% of GE fermented organic waste from watermelon + ripened pineapple peels for 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 4). Also, the Pb concentrations present in the crude oil polluted soil treated with

50% of GE fermented ripen plantain + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 4). However, the levels of Pb in the crude oil polluted soil treated with 70% of garbage enzymes (GE) from fermented organic waste from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 4).

Table 3. Mean and Standard deviation of Cadmium (Cd) Percentage Removal from Polluted Soil Samples Treated with Different Concentration of Garbage Enzyme (GE)

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	1.02 ±0.03[a]	17.13±0.04 [a]	21.83±0.03 [a]	20.44±0.02 [a]	42.03±0.02[a]	42.6±0.01 [a]
Group 2	3.38 ±0.03 [b]	3.75±0.04 [b]	5.94±0.04 [b]	14.25±0.02 [b]	18.36±0.03[b]	25.03±0.03[b]
GE WA+PA (30%)	24.86±0.04 [f]	51.71±0.02 [f]	62.84±0.03	77.23±0.03 [f]	89.08±0.04	92.46±0.05[f]
GE PL+BN (50%)	12.64±0.03 [f]	36.04±0.04 [f]	52.37±0.02	67.39±0.06 [f]	71.05±0.04	89.15±0.02[f]
GE PL+WA+ PA+BN (70%)	14.04±0.03 [f]	37.83±0.03 [f]	51.05±0.05 [f]	63.13±0.03 [f]	83.04±0.04[f]	95.63±0.03[f]
GE PL+WA+ PA+BN (100%)	15.24±.0.04 [f]	18.73±0.03 [f]	44.03±0.04 [f]	53.23±0.04 [f]	72.94±0.03[f]	86.25±0.03[f]

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in means ± Standard Error of Mean (SEM). Values with superscript b are statistically different from group 1 at ($P < 0.05$) down the treatments. Values with superscript f are statistically different from group 1 and 2 at ($P < 0.05$) down and across the treatments.

The concentrations of Pb in the crude oil polluted soil treated with 100% of GE from fermented ripen plantain + watermelon + pineapple + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 4). The percentage garbage enzyme removal of Pb in the crude oil polluted soil sample treated with 30, 50, 70, and 100% ripen plantain, banana, pineapple, and watermelon peel wastes were significantly higher than those of group 1 and 2 (Table 4). The significant percentage of Pb occurrence in the crude oil polluted soil sample after treatment with garbage enzyme fermented from organic peel wastes is indicative GE from fermented organic peel wastes could be potential option for the bioremediation of Pb in soil contamination by lead of increased due to crude oil spill. The percentage removal of Pb obtained from the crude oil polluted soil after treatment using GE from fermented is higher than that reported by Essien *et al.* [24] on the impact of cow dung augmentation for remediation of crude oil polluted Soil by *Eleusine indica*

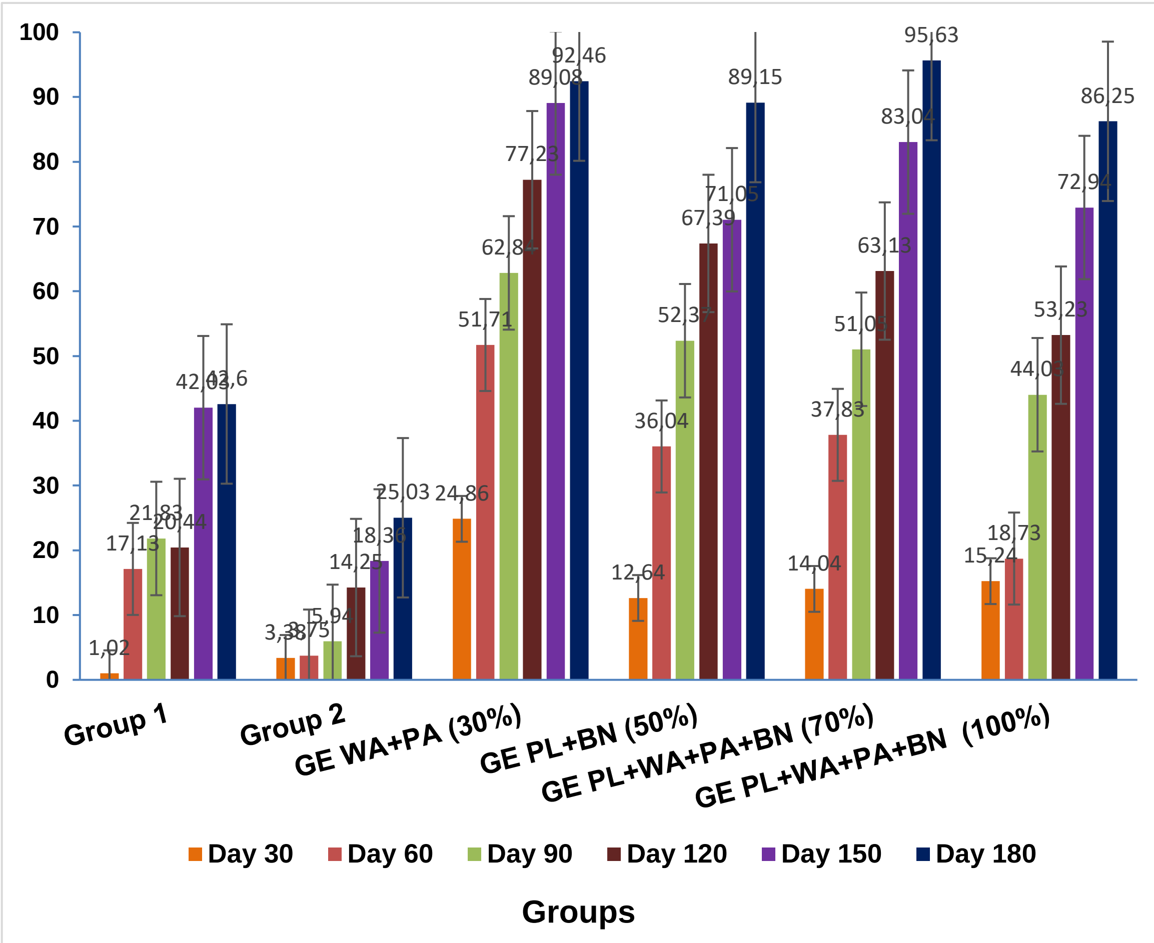

Figure 3. Percentage removal of Cadmium (Cd) in polluted soil samples treated with garbage enzyme (GE).

Table 4. Mean and Standard deviation of Lead (Pb) Percentage Removal from Polluted Soil Samples Treated with Different Concentration of Garbage Enzyme (GE) (n=3)

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	0.54 ± 0.03^{a}	1.72 ± 0.02^{a}	2.57 ± 0.04^{a}	5.25 ± 0.04^{a}	7.03 ± 0.05^{a}	8.04 ± 0.02^{a}
Group 2	0.71 ± 0.02^{b}	0.82 ± 0.02^{b}	0.93 ± 0.02^{b}	2.44 ± 0.03^{b}	2.84 ± 0.03^{b}	3.46 ± 0.03^{b}
GE WA+PA (30%)	0.45 ± 0.04^{b}	2.72 ± 0.03^{f}	6.24 ± 0.03^{f}	10.35 ± 0.03^{f}	17.84 ± 0.03^{f}	25.74 ± 0.02^{f}
GE PL+BN (50%)	2.34 ± 0.04^{f}	20.55 ± 0.04^{f}	27.63 ± 0.02^{f}	34.84 ± 0.03^{f}	45.04 ± 0.04^{f}	50.34 ± 0.04^{f}
GE PL+WA+ PA+BN (70%)	9.84 ± 0.04^{f}	15.73 ± 0.03^{f}	24.04 ± 0.04^{f}	37.82 ± 0.02^{f}	43.74 ± 0.03^{f}	61.04 ± 0.04^{f}

GE PL+WA+ PA+BN (100%)	7.03 ±0.02 f	11.81±0.01 f	21.65±0.03 f	29.55 ±0.04f	47.32 ±0.02 f	50.63 ±0.03f

GE = garbage enzymes, WA = watermelon, PA=pineapple, PL = plantain, BN = banana. Values are reported in means ± Standard Error of Mean (SEM). Values with superscript b are statistically different from group 1 at (P < 0.05) down the treatments. Values with superscript f are statistically different from group 1 and 2 at (P < 0.05) down and across the treatments.

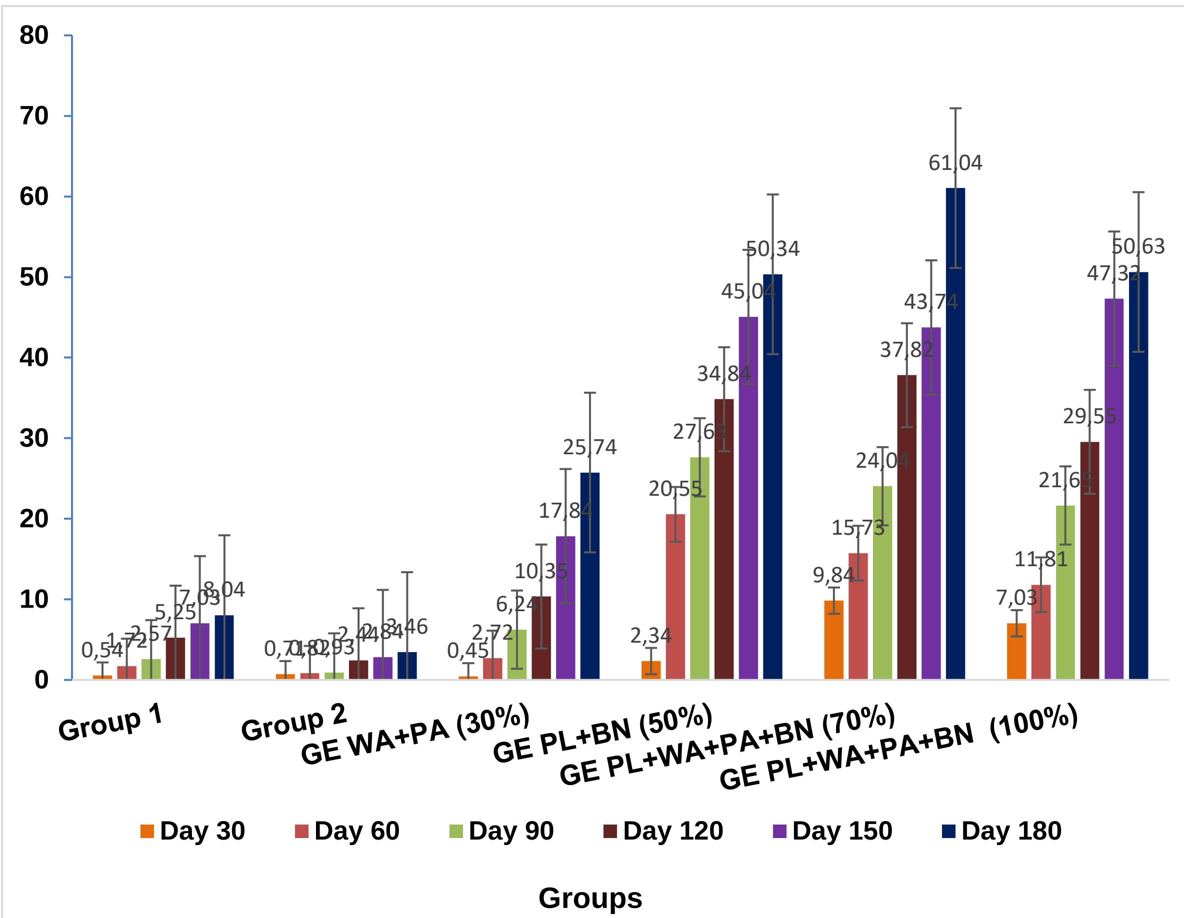

Figure 4. Percentage removal of Cadmium (Cd) in polluted soil samples treated with garbage enzyme (GE).

Table 5 shows the percentage removal of nickel (Ni) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The Ni levels in the crude oil polluted soil treated with 30% of GE fermented organic waste from watermelon + ripened pineapple peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 5). The Ni concentrations present in the crude oil polluted soil treated with 50% of GE fermented ripen plantain + banana waste peels for 30, 60, 90, 120, 150, and

180 days were significantly higher than those of group 1 and 2 (Table 5). More so, the levels of Ni in the crude oil polluted soil treated with 70% of garbage enzymes (GE) formulated from fermented organic waste from ripen plantain + watermelon + pineapple + ripen banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 5).

The concentrations of nickel (Ni) in the crude oil polluted soil treated with 100% of GE fermented from ripen plantain + watermelon + pineapple + banana waste peels for 30, 60, 90, 120, 150, and 180 days were significantly higher than those of group 1 and 2 (Table 5).

The percentage GE removal of Ni in the crude oil polluted soil sample treated with 50% GE from fermented watermelon and pineapple peel wastes for 30, 60, 90, 120, 150, and 180 days were significantly higher than those treated with 30% of GE from fermented organic peel wastes of watermelon and pineapple peel wastes, and 100% GE from ripen plantain, banana, watermelon, and pineapple peel wastes (Table 5). The significant percentage removal of Ni observed on crude oil polluted soil sample after treatment with GE from fermented organic peel wastes at 30, 50, 70, and 100% for 30, 60, 90, 120, 150, and 180 days are reflective of the Ni decontamination potential of GE from fermented watermelon, pineapple, ripen plantain, and banana peel wastes against soil toxicity due to high Ni levels, hence might perhaps be an alternative tool of bioremediation of high Ni levels in soil arising from crude oil pollution. The result obtained on the percentage Ni removal of GE from fermented organic peel wastes is in line with the report of Uwazie *et al.* [25] on the remediation ability of melon grass in a crude oil polluted soil in a tropical region.

Table 5. Mean and Standard Deviation of Nickel (Ni) Percentage Removal from Polluted Soil Samples Treated with Different Concentration of Garbage Enzyme (GE) (n=3)

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	2.34 ± 0.03^{a}	3.52 ± 0.02^{a}	6.14 ± 0.03^{a}	9.87 ± 0.04^{a}	13.34 ± 0.03^{a}	18.24 ± 0.02^{a}
Group 2	1.65 ± 0.04^{b}	2.43 ± 0.03^{b}	4.04 ± 0.03^{b}	6.74 ± 0.02^{b}	26.73 ± 0.04^{b}	36.02 ± 0.02^{b}
GE WA+PA (30%)	7.94 ± 0.04^{f}	13.16 ± 0.03^{f}	17.02 ± 0.04^{f}	22.63 ± 0.04^{f}	27.45 ± 0.02^{f}	38.83 ± 0.04^{f}
GE PL+BN (50%)	9.42 ± 0.04^{f}	28.54 ± 0.04^{f}	51.42 ± 0.04^{f}	57.25 ± 0.03^{f}	71.02 ± 0.04^{f}	83.04 ± 0.04^{f}
GE PL+WA+PA+BN (70%)	4.62 ± 0.04^{f}	7.23 ± 0.02^{f}	11.54 ± 0.03^{f}	22.03 ± 0.03^{f}	29.83 ± 0.03^{f}	52.04 ± 0.03^{f}
GE PL+WA+PA+BN (100%)	8.03 ± 0.04^{f}	16.42 ± 0.04^{f}	23.94 ± 0.02^{f}	32.82 ± 0.01^{f}	42.63 ± 0.03^{f}	51.82 ± 0.02^{f}

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in means ± Standard Error of Mean (SEM). Values with superscript b are statistically different from group 1 at ($P < 0.05$) down the treatments. Values with superscript f are statistically different from group 1 and 2 at ($P < 0.05$) down and across the treatments.

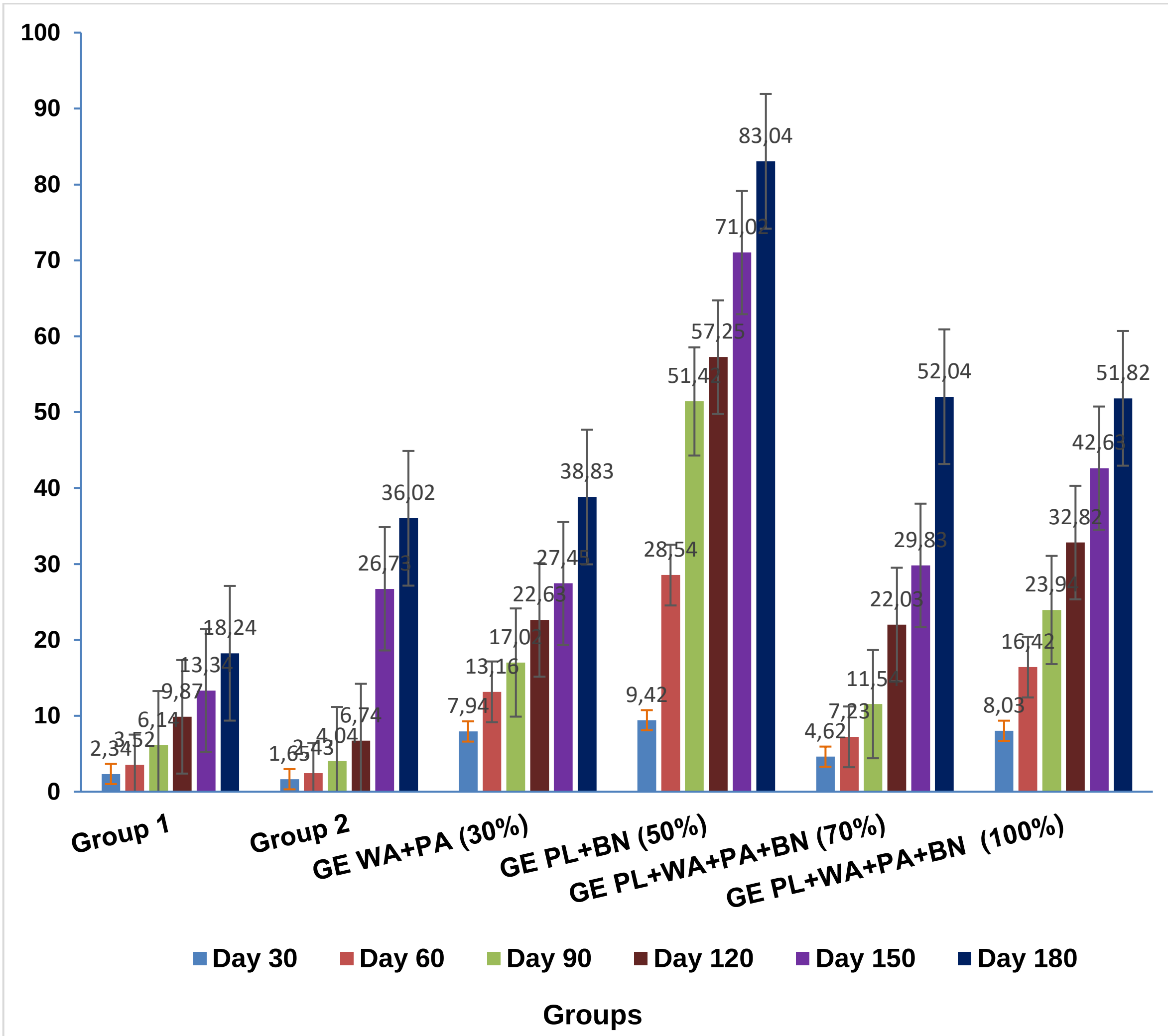

Figure 5. Percentage removal of Cadmium (Cd) in polluted soil samples treated with garbage enzyme (GE).

4. CONCLUSION

Soil contamination is a serious global issue. For this reason, good and effective bioremediation technology is of great necessity. In this research, garbage enzymes ferments from ripen plantain, banana, watermelon, and pineapple peel wastes at varying percentage were used to treat crude oil polluted soil samples from 30-180 days. Garbage enzymes from fermented organic peel wastes at 30, 50, 70, and 100% significantly elicited percentage removal of Pb, Cd, Hg, Ni, and Cr from crude-induced soil toxicity. Therefore, using garbage enzymes from fermented organic peel wastes in the bioremediation of toxic heavy metals in oil-contaminated soils could play a crucial role in soil restoration and also provide peel wastes management as well as economic benefits for the soil bioremediation projects. However, it is important to note that this research was conducted on a laboratory scale, and further studies are necessary to evaluate the effectiveness of these methods on a larger scale.

Note

This research displayed the effectiveness of garbage enzyme mixture from fermented organic peel wastes in the decontamination of selected heavy metals in crude oil contaminated soil. This concept should be carefully investigated in the light of current pollutants remediation techniques so that they can be accepted as appropriate bioremediation tools.

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