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Article

Bioremediation of Heavy Metals in Crude Oil-Contaminated Utisol, Using Nutrient Formulate Produced from *Jatropha tanjorensis* Leaf Extract

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Abstract: This work evaluated the bioremediation potential of *Jatropha tanjorensis* leaf extract at different masses (250g, 500g and 750g) over a 40-day period. To achieve this, crude oil contamination of sandy loam soil was stimulated in twelve plastic reactors containing fixed masses of soil (4kg each) of topsoil homogenized with 500g of Bonny light crude oil. The *Jatropha tanjorensis* leaves were cultivated, rinsed with distilled water, blended, and purified by filtration. The leaf extract was applied at the stated concentrations including a control reactor (without leaf extract). The plastics reactors were kept in an open air shielded away from rainfall. The physicochemical characteristics determined were particle size distribution (PSD), potential of hydrogen (pH), electrical conductivity (EC), organic matter (OM), organic carbon (OC), selected heavy metals (Cr, Cd, Zn, Pb) and sample management were all in line with standard procedure. After 40 days of treatment, results obtained showed that plastic reactor with 750g of leaf extract produced the highest amount of cadmium reduction of 97% (from an initial of 3.3×10^{-2} to 9×10^{-4}mg/kg), and there was significant difference among treatment ($P < 0.05$). The sequence of reduction among treatment was 750g > 500g > 250g of the leaf extract. Chromium, Lead and zinc followed similar trend. Thus, the *Jatropha tanjorensis* leaf extract has the potential to ameliorate crude oil-contaminated soil.

Keywords: Hospital Too Far; Biostimulation; Contamination; Euphorbiaceae

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1. Introduction

The predictable release of crude oil resulting from corrosion and vandalization of pipelines has in recent times become one of the major global environmental concerns especially in the Niger Delta region of Nigeria as a result of its hazardous nature. Records show the negative impact of crude oil on soil [1], which is known to be one of the earth's most important natural resources [2], as any nation that destroys its soil has destroyed itself [3]. The importance of soil is enormous as it provides a growing medium for plant, roots with nutrients and minerals, exchange of oxygen and other relevant gases, protection from erosion and speeds up natural decomposition process of organic matter. Thus, healthy soil is crucial for the wellbeing of humans, animals, and plants [4].

Huge amount of crude oil gets released and causes heavy metals (HMs) pollution to the nearby vicinities thereby creating severe damage to the ecosystems and massive health issues to the organisms including human beings [5]. The soil, being the ultimate part of the ecological system, is profoundly polluted by crude oil associated heavy metals [6]. Moreover, heavy metals persist in the soil for a long time due to their adherent quality and therefore the soil is considered the major sink for HMs [7].

Polluted soil can be reclaimed through remediation. Remediation of petroleum hydrocarbon contaminated sites can be achieved through physical (e.g., incineration, and

disposal in landfills), chemical (use of chemical oxidants) and biological process (use of plants) [8]. Biological treatment, commonly referred to as bioremediation involves the breakdown of contaminants into non-toxic forms through the activities of micro-organism [9]. It has been canvassed and adopted over chemical treatment methods because it is cost-effective and eco-friendly [10].

Thus, there is necessity for careful selection of the most suitable analytical method for evaluating petroleum hydrocarbon contaminated soils. The adoption of living organism would efficiently degrade or detoxify environmental contaminants into less toxic forms [11]. Bioremediation techniques are typically more economical than traditional methods such as incineration [12]. Several bioremediation technologies have been resorted to, in a bid to affect the clean-up of contaminated soils. One of such technologies is biostimulation [13, 14]. In this technique, the growth of native microbial populations in contaminated soil is stimulated by providing essential nutrients for optimal pollution treatment [10]. The technique is cost-effective, long-term, environmentally, and aesthetically friendly method of immobilizing/ stabilizing and transferring contaminants such as pesticides, metals, and chlorinated hydrocarbons into innocuous substances without posing any threat to the environment [15].

Several plant leaf extracts with remediation potential have been utilized to recover crude oil contaminated sites. Some plants have more potential to remediate contaminated soil than the others. *Jatropha tanjorensis* J.L.Ellis & Saroja, commonly called hospital too far, catholic vegetable, iyang- ipapa lapalapa [16] is a perennial herb that belongs to the *Euphorbiaceae* family. The plant is used for medicinal and nutritional purposes. It grows like weed in great quantity in the Niger Delta region of Nigeria (Figure 1), despite its medicinal importance.

Figure 1. *Jatropha tanjorensis* plant.

There is dearth of literature on the potential of *J. tanjorensis* leaf extract in the reclamation of petroleum hydrocarbon contaminated soil especially in West Africa countries, where the leaf extract is discarded as waste. This plant knowledge is important, as it would help inform the choice of suitable bioremediation agent that can be used to remediate crude oil contaminated soil and as well provide a cost effective and environmentally friendly approach.

Several related studies have been carried out such as nutrient, phytochemical and anti-nutrient composition of *J. tanjorensis* leaf extract [17] antibacterial screening of the extracts [18] anti-anaemic effect of aqueous and methanolic leaf extracts [19]. However,

bioremediation potential of the leaf extract in crude oil - contaminated soil investigations have not been widely considered. This work, seeks to investigate the bioremediation potential of *J. tanjorensis* leaf extract in crude oil-contaminated sandy loam soil.

2. Materials and Methods

2.1. The Study Area

The study was conducted at the research farm of the Rivers State Institute of Agricultural Research and Training, which is situated in the Rivers State University, Port-Harcourt, Nigeria as shown in Figure 2, on a global positioning system (GPS) coordinate of latitude 4° 53′ - 4° 62′ E and longitude of 6° 58′- 7° 54′ N, with a predominance of oxisols (United States Department of Agriculture (USDA) soil taxonomic order) [1] and its soil texture is sandy loam soil. Rivers state (Figure 2) is characterized by tropical rainforest vegetation with rainfall ranging from 2000 – 2484mm per annum of which 70% occurs between the months of May and August with an average temperature of 27°C [14, 20].

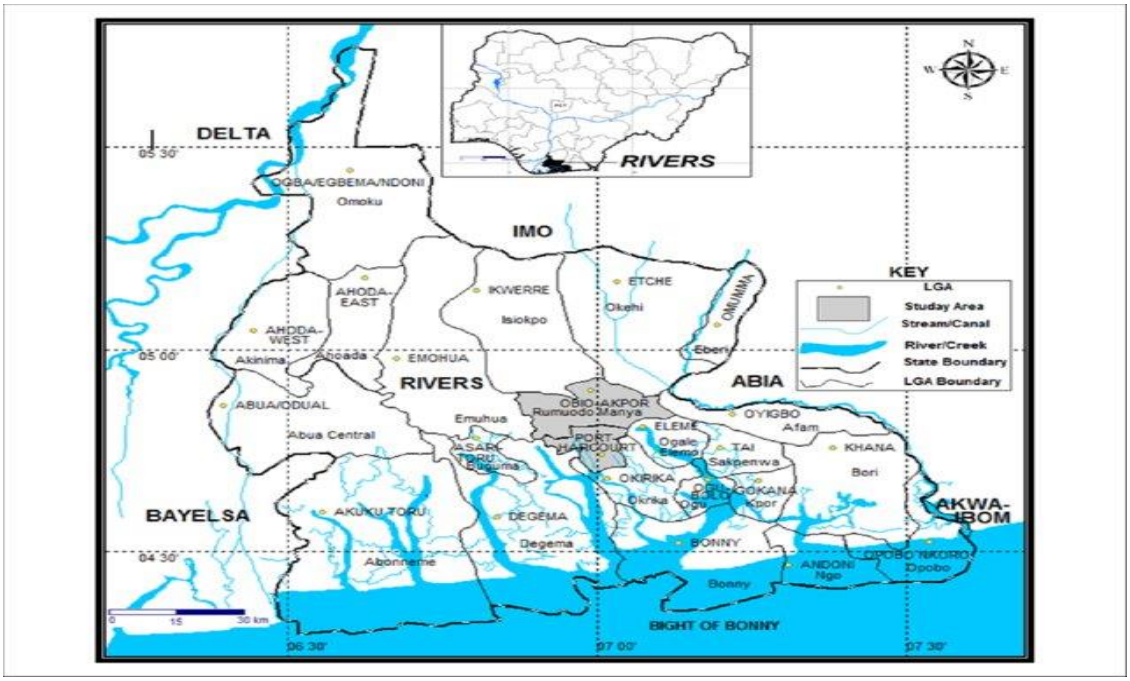

Figure 2. The Study Area, Rivers State, Nigeria.

2.2. Samples Collection and Treatment

Soil samples were collected from the soil before mixing with crude oil. The samples were also collected prior to petroleum-contamination of the soil, and every 10 days until the end of the study period for laboratory analysis to determine the residual heavy metals content for a period of 40 days. The Bonny light crude oil sample was collected from Shell petroleum development company (SPDC), using plastic reactor. Each plastic reactor was contaminated uniformly with 500 g of crude oil. The cylindrical plastic container (reactor) with 0.3m diameter and 0.35 m depth were employed to accommodate the mixture of soil and crude oil as well as the amendment (*J. tanjorensis* leaf extract) that provides micro-organisms with nutrient to enhance microbial activities in the contaminated soil. Thus, degrades contaminant in the soil into less toxic or non-toxic form [21]. The soil was measured using a weighing balance.

The reactors were kept in an open air but shielded from rain to enable direct control of nutrient and moisture levels to prevent excessive run-off of the contaminant [22]. The

reactors were left undisturbed for three days to allow for adequate infiltration and percolation of the contaminant. The treatment was applied after a three-day period. Each of the reactor received 100 ml of water through a perforated can at three days interval all through the study period, the application rate was in line with previous studies that showed its effectiveness in remediation of oil-polluted soils [23, 20].

2.3. Preparation of Jatropha tanjorensis leaf extracts

The leaf samples of *J. tanjorensis* were cultivated from Rivers state institute of agricultural research and training, situated in Rivers state university, Port-Harcourt. The samples were washed several times and rinsed with distilled water. Then, it was placed into a portable electric blender. After blending, the solution obtained was purified by filtration using whiteman® No 1 filter paper [24] and further sterilized by filtration through a Millipore® membrane filter of 0.45 μm pore size [25]. The liquid extracts were stored in sterile capped bottles and refrigerated until use.

2.4. Experimental design

Single factor experiment in a randomized complete block design (RCBD) was used in this study. The treatment consists solely of the different levels of single variable factors. All other factors are uniformly applied in all reactors at a prescribed level [26]. The treatment consisted of various masses of leaf extract (0 g, 250 g, 500 g, and 750 g). All other factors such as the quantity of soil and crude oil were applied uniformly to all plastic reactors at a single prescribed level as shown in Table 1. The experimental design consisted of 4 treatments with 3 replicates, each making a total of 12 treatment reactors for crude oil contaminated soils. They were labelled B_1, B_2, B_3, including the control (B_4) (See Table 1). Randomizations was achieved using the draw lot approach as described in several literatures [26].

Table 1. Experimental mix proportion

Reactor	Mix proportion	Mix ratio.
B_1	4000g of soil + 500g of crude oil + 250g of leaf extract	8:1:0.5
B_2	4000g of soil + 500g of crude oil + 500g of leaf extract	8:1:1.0
B_3	4000g of soil + 500g of crude oil + 750g of leaf extract	8:1:1.5
B_4 (Control)	4000g of soil + 500g of crude oil + 0g of leaf extract (Control)	8:1:0.0

2.5. Sampling and Analytical Methods

This study did not consider the determination of heavy metal levels in crude oil used for sandy loam soil pollution simulation because no effect since the initial concentration of heavy metals in the treatment process was identified in the polluted soil and not the crude oil [27]. Prior to and after contamination of the soil using crude oil, composite soil samples were collected for analysis. The physicochemical properties of the uncontaminated soil determined were particle size distribution (PSD), potential of hydrogen (pH), moisture content (MC), electrical conductivity (EC), organic matter (OM), organic carbon (OC) and heavy metals (HMs) including cadmium, chromium, zinc and lead. After contamination, heavy metals were determined at intervals of 10 days for a period of 40 days. The moisture content (MC) of the soil was determined by the 24-hour oven-drying method, particle size distribution (PSD) was determined using hydrometer method while the texture was ascertained by the United State Department of Agriculture (USDA) soil textural classification scheme with the aid of TALS for window computer software 4.2. A handheld meter (H198331) known as Hanna instrument was used to carry out ***in-situ*** measurement for pH and EC. The organic carbon and organic matter were determined by the Walkley-black combustion method. The Heavy metals were

determined by flame atomic absorption spectrometry (AAS). The percentage of heavy metal removal was deduced using equation 1.

$$\textbf{HM Removal } (\%) = \frac{\textbf{IC}-\textbf{FC}}{\textbf{IC}} \textbf{ X 100} \quad (1)$$

Where HM is heavy metal
IC is initial concentration of heavy metal (mg/kg)
FC is final concentration of heavy metal (mg/kg)

2.6. Statistical Analysis

The mean, and standard error were determined using AVERAGE and Standard Error functions, respectively in the Microsoft Excel 2016© as well as simple percentage. Data were analyzed using analysis of variances (ANOVA) following the method of [26] to determine if there are statistically significant differences at 5% significance level.

3. Results

3.1. Physicochemical Properties of the uncontaminated Soil

The key physicochemical characteristics of the uncontaminated soil used to house the crude oil and treatments. The particle size analysis of the uncontaminated soil sample consisted of 59.0% sand, 28.6% silt and 12.4% clay, indicating that the soil texture is sandy loam soil as shown in Figure 3, electrical conductivity (EC) was 17.1µS/cm, 0.69% organic carbon (OC), 0.89% organic matter (OM), 8.91% moisture content (MC) and pH value was 5.82 which reveals the acidity of the sandy loam soil (Table 2).

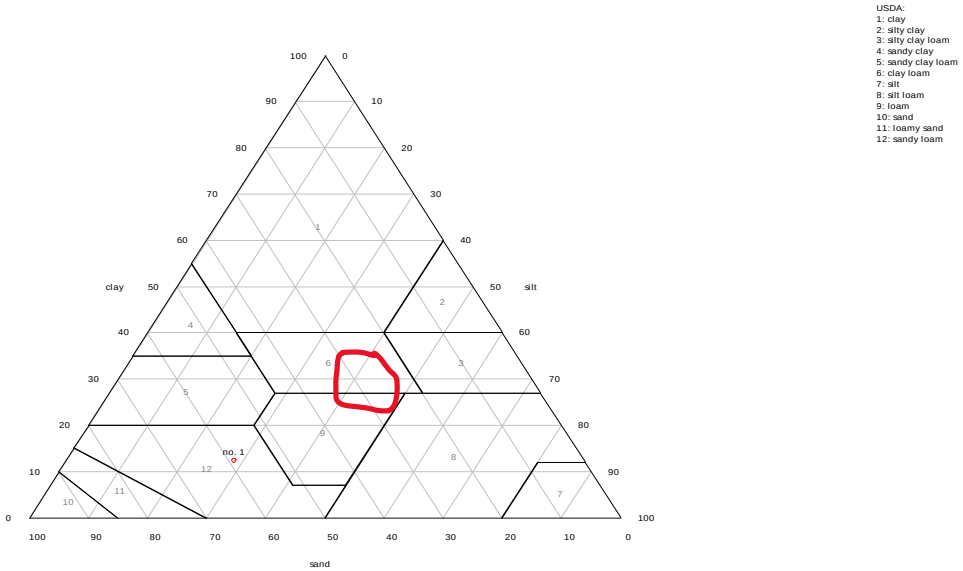

Figure 3. Soil Textural Triangle Showing Sandy Loam (No. 12) as the texture of the soil used in this study based on the USDA Soil Textural Classification Scheme as Determined using TAL® for Windows v. 4.2 (Christopher Tech Boon Sung, China).

Table 2. Physical and Chemical Properties of the Uncontaminated Soil

Sand (%)	Silt (%)	Clay (%)	MC (%)	pH	EC (µS/cm)	OC (%)	OM (%)	Cd (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
59	28.6	12.4	8.91	5.82	17.1	0.69	0.89	ND	ND	ND	ND

MC, Moisture Content; EC, Electrical Conductivity; OC, Organic Carbon. OM, Organic Matter; ND, Not Detected.

4. Discussion

4.1. Chemical Characteristics of the Untreated Crude oil Contaminated Soil

Figure 4 shows the chemical characteristics of the petroleum hydrocarbon-contaminated soil. The crude oil contaminated sandy loam soil contains heavy metals. Previous reports have established that crude oil contains several inorganic and carcinogenic pollutants such as heavy metals that cause heavy risks to the surrounding environment [27-29]. It was further observed as shown in Figure 4, that cadmium concentration ranged from 0.033 to 0.034 mg/kg; 0.120 to 0.121 mg/kg for chromium; 0.686 to 0.689 mg/kg for lead, and 0.78 to 0.79 mg/kg for zinc concentrations of the untreated soils including the control which were far below the regulatory value of 12 mg/kg, 380 mg/kg, 530 mg/kg and 720 mg/kg for Cd, Cr, Pb and Zn, respectively, as spelt out by [30] across all treatments. However, the concentration of the heavy metals was quite low (Figure 4) but can still cause harm to human health.

This therefore justifies the need for remediation of the petroleum hydrocarbon-contaminated soils before it can finally be utilized for agricultural purposes or reuse for civil works. It is important to note that there was little variation of the selected heavy metals (cadmium, chromium, lead, zinc) content after contaminating the soil with 500 g of crude oil across all reactors (Figure 4).

4.2. Remediation of crude oil-contaminated soil over time

The average cadmium concentration in the contaminated media 3 days after mixing the soil and crude oil was 0.033 mg/kg (Figure 4). This corresponds to the 3 days after contamination (3DAC) in the graph (Figures 4 and 5). The contaminant concentrations comprise of soil, crude oil and amendments.

After 10 days, there were significant drop in cadmium, which varied across different treatments. The cadmium reduces in treatments B_1, B_2 and B_3 with 250 g, 500 g and 750 g of *J. tanjorensis* leaf extract, respectively, there were significantly different from each other ($P < 0.05$) and it fell within 79 to 84% reduction range. In contrast, the contaminated soil with 0 g of the leaf extract, which is referred to as the control (B_4) increased by 3%. After 10 days of applying treatment, the cadmium in reactor B_3 decreased to 5.1×10^{-3} mg/kg, and this corresponded to 84.5% cadmium reduction (Figure 4 and 5). There were significant differences ($P < 0.05$) in the cadmium content across all the treatments except for the control. The contaminant decreased with time, at a fast rate in the first 30 days and became slower afterwards. This agrees with the findings of [27]. However, it's obvious that heavy metals cannot be degraded during bioremediation but can be transformed from one organic complex or oxidation state to another. Heavy metals can transform to either less toxic, easily volatilized or less bioavailable due to a change in their oxidation state [31].

At 40 days after treatment (Figure 4), the cadmium reduction percentage were 90, 91, and 97% for treatments B_1, B_2, and B_3 with 250 g, 500 g and 750 g of *J. tanjorensis* leaf extract, respectively. It is evident from Figure 5 that the cadmium reduction was highest in soil treated with 750 g *J. tanjorensis* leaf extract (B_3) (decreased from an initial 3.3×10^{-2} to 9×10^{-4} mg/kg), followed by B_2, B_1 and B_4. The cadmium content decreased far below the local regulatory target value of 0.8 mg/kg as prescribed by [30] for soil and sediment. This confirms the findings of [32] stating that microbes could remove small amounts of metals from the soils as low metal concentrations can stimulate microbial activity in soils.

The concentration of Cr over time are shown in Figure 4. After 10 days, the chromium concentration was reduced to 47 to 60% in all the treatment except for the reduction level in the control reactor, which was quite low (2.5% reduction). There was significant difference in all treatment including the untreated contaminated soil ($P < 0.05$). At the 40th day, the treatments with 750 g of *J. tanjorensis* leaf extract (Treatment B_3) showed the least

and highest chromium concentration of 3.2×10^{-2} mg/kg and 74% reduction, respectively.

The Average Pb concentration in the contaminated media 3 days after mixing the soil and crude oil was 0.69 mg/kg (Figure 4). After 10 days, Pb dropped, which varied across different treatments. The Pb reduces in treatments B_1, B_2 and B_3 with 250 g, 500 g and 750 g of *J. tanjorensis* leaf extract, respectively, were significantly different from each other ($P < 0.05$) and fell within 31 to 53% range. In contrast, the contaminated soil with 0 g of the leaf extract, which is referred to as the control (B_4) increased by 2%. After 10 days after applying treatment, the Pb in treatment B_3 decreased to 0.32 mg/kg, corresponds to 53% Pb reduction (Figure 4 and 5). There were significant differences ($P < 0.05$) in the Pb content across all the treatments.

The concentration of Zn over time are shown in Figure 4. After 10 days, the Zn concentrations were reduced to 43 to 47% in all the treatment except for the reduction level in the control container, which was quite low (1.4% reduction). There were significant differences in all treatment including the untreated contaminated soil ($P < 0.05$). After 40 days of treatment, 750 g of *J. tanjorensis* leaf extract (Treatment B_3) had better performance, it dropped from 0.78 to 0.28 mg/kg (64% reduction). However, the Zn reduction in the control container was the lowest (3.6% reduction). Thus, the observed results seem to suggest that high amendment dosage can accentuate the rate of initial HMs biodegradation and then reduce the remediation period of soil contaminated with crude oil [33]. Similar observations have been documented [34-36].

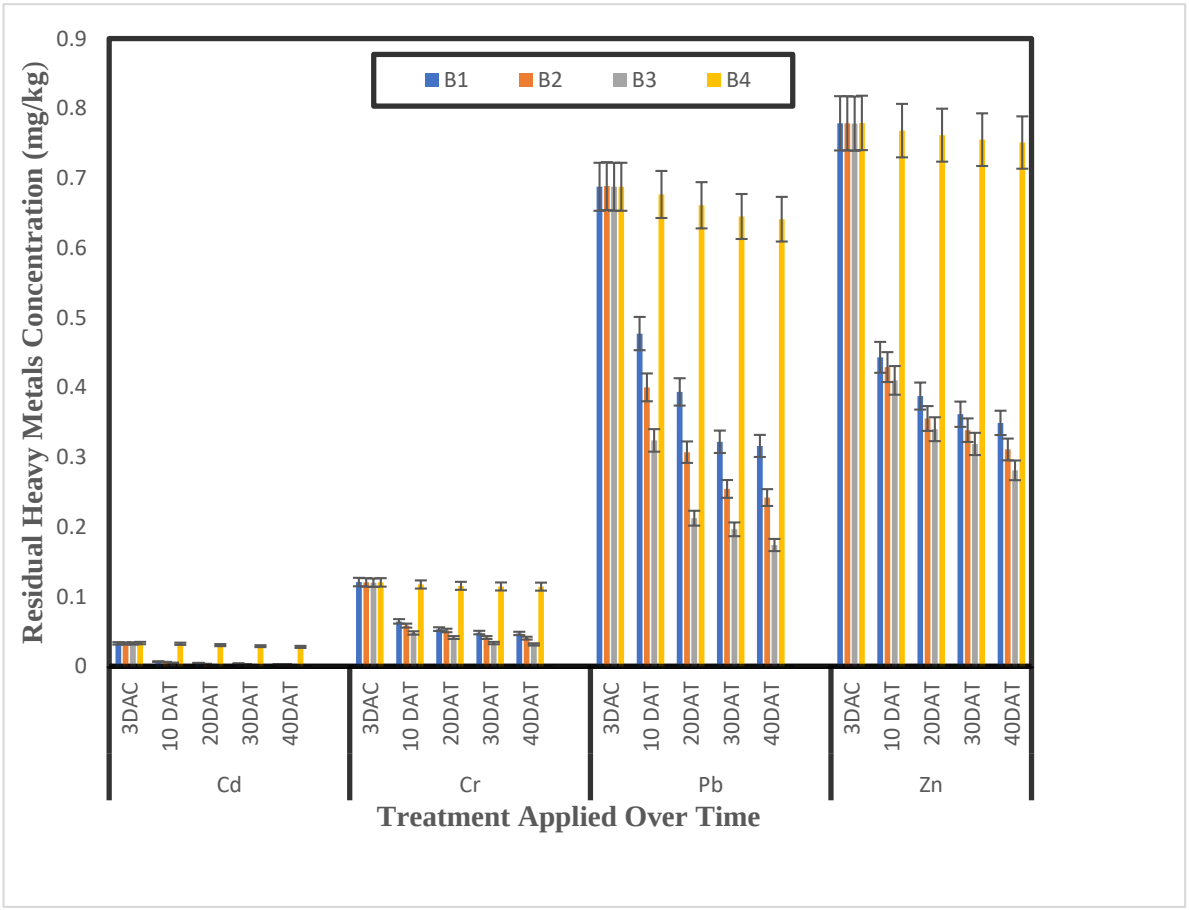

Figure 4. Comparison of Heavy metals among the different matrixes in the reactors over time in crude oil-contaminated sandy-loam soil. [DAC = days after contamination, DAT = days after treatment, B_1 = 250g of leaf extract, B_2=500g of leaf extract, B_3 =750g of leaf extract, B_4= 0g of leaf extract (Control); Error bars on chart are percentage]

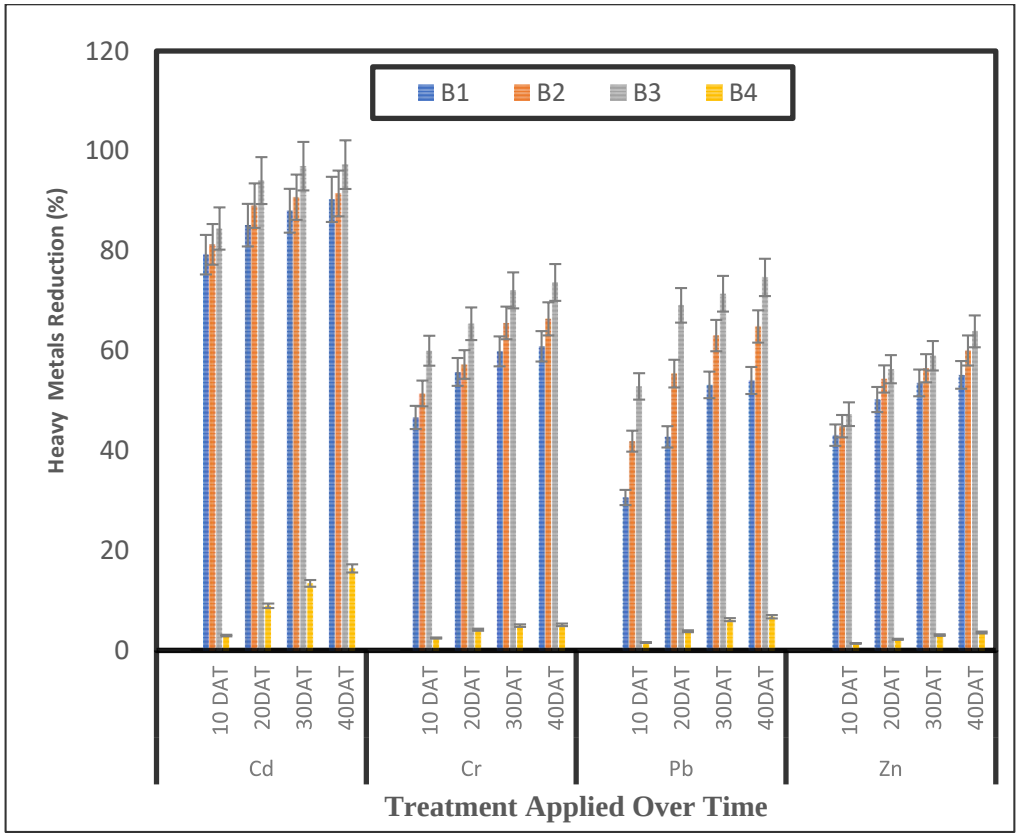

Figure 5. Comparison of Heavy metals (In terms of Heavy Metals Reduction) over time in crude oil-contaminated sandy-loam soil. [DAC = days after contamination, DAT = days after treatment, B_1 = 250g of leaf extract, B_2=500g of leaf extract, B_3 =750g of leaf extract, B_4= 0g of leaf extract (Control); Error bars on chart are percentage]

5. Conclusions

The biological remediation potential of *J. tanjorensis* leaf extract over a 40-day period, with a view of assessing the suitability of remediating heavy metals in crude oil-contaminated sandy loam soil. Results obtained showed that 750 g of *J. tanjorensis* leaf extract (Treatment B_3) was the most suitable for the removal of heavy metals in crude oil-contaminated soils, accounting for a cadmium reduction of 97% after 40 days period of remediation. The sequence of the cadmium reduction by different treatments was $B_3 > B_2 > B_1 > B_4$. Cr, Pd and Zn followed similar trend. Thus, the *J. tanjorensis* leaf extract has the potential to remove heavy metals in crude oil-contaminated soils.

Author Contributions: individual contributions "Conceptualization, U.B.N.; and D.Q.E.; methodology, C.E.; formal analysis, D.Q.E.; investigation, U.B.N.; resources, D.Q.E..; data curation, C.E.; writing—original draft preparation, D.Q.E.; writing—review and editing, U.B.N.; supervision, U.B.N.

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