International Journal of Innovative Agriculture & Biology Research 12(1):87-96, Jan.-Mar., 2025

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doi:10.5281/zenodo.15260337

Soil Remediation: The Potentials of *Leuceana leucocephala* (Lam.) De Wit in Crude Oil-polluted Soil Ecosystem Restoration And Re-vegetation

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ABSTRACT

The adverse effects of crude oil and its derivatives on soil is enormous. Phytoremediation is a cheaper and sustainable method of addressing this menace. Therefore, the remediating potential of *Leuceana leucocephala* (LL) on crude oil-contaminated soil was investigated in Wilberforce Island, Nigeria. Varying concentrations of crude oil (0, 25, 50, 75, and 100 ml) were applied to LL seeds (n=10) in Petri dishes arranged in completely randomised design (r=5). Germination Percentage-GP was determined at day 10. Two weeks after contamination, LL seedlings were planted in plant bags (containing oil-contaminated soil) and arranged in completely randomised design (r=10). Plant Height-PH (cm), Plant Girth-PG (mm) were determined at 4, 8, 12 and 16 Weeks After Planting-WAP while Number of Leaves-NL and Number of Nodules-NN at 16 WAP only. The physicochemical parameters of the soil were determined following standard procedures. The LL seedlings contaminated with 75 and 100 ml crude oil had GP of 62 and 56%, respectively which were significantly lower than 92% (control). At 16 WAP, the PH and PG of LL seedlings ranged from 41.80±6.50 (100 ml) to 58.30±4.75 (25 ml) and 0.19±0.04 (100 ml) to 0.34±0.03 (25 ml) respectively, which were significantly lower than 73.70±4.40 and 0.54±0.03 (control). The NL and NN of LL seedlings ranged from 6.00±2.00 (100 ml) to 16.00±2.00 (25 ml) and 2.00±1.00 (100 ml) to 10.00±2.00 (50 and 25 ml) respectively, which were significantly lower than 24.00±3.00 and 18.00±3.00 (control) at 16 WAP. The pH of the polluted soil ranged from 5.22 (0 ml) to 5.03 (100 ml) when compared to an aggregate of 5.72–5.31 (control). Although there was a decline in the attributes of the growth parameters, *L. leucocephala* was able to endure in crude oil contaminated soil. The tolerance of *L. leucocephala* in crude oil-contaminated soil may indicate the soil rehabilitation and re-vegetation potential of *L. leucocephala* in the oil-rich area of Nigeria.

Keywords: Phytoremediation, crude oil, leguminous plant species, polluted soil.

INTRODUCTION

Crude oil is among the major energy resources on global perspective. Due to its importance in industrial processes and its role in global trade, crude oil is unquestionably the most significant and widely traded commodity (Muhammad et al., 2019). Exploration of crude oil is still an emerging issue especially in major oil producing nations. As a result of this, both living and non-living entities of the environment have been exposed to great danger.

In some regions, severe pollution has been attributed to crude oil spillage, toxicity and its complex nature (Oyedeji et al., 2022). According to Gao et al. (2022), nowadays, organic and inorganic compounds derived from crude oil are polluting a number of areas around the world. This is due to modern civilization, urbanization and mechanization development, leading to excessive dependance on petroleum and petroleum-based products. Petroleum hydrocarbons from crude oil bioaccumulates in humans and biomagnifies in the environment. According to Laffon et al. (2016), hydrocarbons can bioaccumulate in aquatic species exposed to oil spills, and is subsequently transferred to humas through the food chain. Oil spillages introduce non-organic pollutants that are toxic, carcinogenic, mutagenic, and teratogenic, and hence pose serious threat to living things (Yap et al., 2021). Previous studies of Wang *et al*. (2017), Kayode and Oyedeji (2012), Oyedeji et al. (2012), Oyedeji et al. (2015), Truskewycz *et al*. (2019) Grosell and Pasparakis (2021), King et al. (2021) have all confirmed the detrimental impacts of crude oil on biotic and abiotic entities in the ecosystems.

The adverse effects of crude oil and its derivatives on soil is enormous. This is further aggravated by the alarming rate of oil theft in some oil producing areas. According to Kalejaye (2015), the Nigerian oil and gas sector has recorded an alarming 9000 instances of spillage annually, these were reported by the Nigerian oil and gas sector. Furthermore, in Nigeria's Niger Delta region, oil spills and environmental degradation are largely caused by oil pipeline vandalism (Iwegbue et al., 2021). It is important to note that contamination of soil by crude oil and its derivatives has negative impact on the environment and often takes substantial time to amend (Kayode and Oyedeji 2012; Oyedeji et al., 2012; Oyedeji et al., 2015),. There is need to clean up this pollution because of its deleterious effects on human health, plants, animals and the environment.

There are a number of drawbacks to the mechanical and physiochemical-based remediation techniques often employed to clean up crude oil contamination in soil and water, including their astronomical cost and potential for ecosystem degradation (DalCorso et al., 2019). Hence, phytoremediation has subsequently been widely encouraged. This technique uses plants and its rhizospheric activities to get rid of pollutants or make them less bioavailable to the ecosystem (Yan et al., 2020). The technique is about 90% cheaper when compared to other conventional techniques (Zahra et al., 2022).

Typically, phytoremediation of crude oil sometimes requires amendment of the soil and linked microorganisms in the root complex. According to Zahra et al. (2022) some plants can remediate contaminated soil with the aid of their root systems. These root complexes create a microbiome for contaminants' buildup and regulate their bioavailability (DalCorso et al., 2019).Scientists have worked on phytoremediation potentials of some plant species on crude oil contaminated soils. These include *Glycine max* (Njoku et al., 2009), *Albizia lebbeck* (Oyedeji et al., 2021) and *Albizia procera* (Oyedeji and Oyedeji, 2022). Although, the efficacy of *Leucaena leucocephala* in the remediation of crude oil polluted soil was reported by Edwin-Wosu (2011), there is still paucity of data on its potential in remediating soil contaminated by crude oil in Nigeria. This necessitates the need to examine the plant as a potential phytoremediator in Nigeria.

MATERIALS AND METHODS

Study sites

The investigations were conducted at the Screen House, Department of Biological Sciences and Central Research Laboratory, Faculty of Science both at Niger Delta University, Wilberforce Island, Nigeria. The physicochemical analyses of soil samples were done at the Central Research Laboratory of Federal University of Technology, Akure, Nigeria.

Source of experimental samples

Clay-loamy top soil was taken at a depth 0 – 10 cm at the Research and Experimental Farm, Niger Delta University, Wilberforce Island, Nigeria. Furthermore, the crude oil (Bonny light crude oil) was obtained from Oporoma Flow Station of Shell Petroleum Development Company. The plant (*Leuceana leucocephala*) was sourced from Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Viability test of the seeds used

In this investigation, the Floating technique from Anoliefo and Vwioko (1995) was used. A beaker of distilled water was used to soak about 500 seeds of the plant species for a total of 30 minutes. Exactly 300 viable seeds were chosen at random from among the seeds that were submerged in the water and the seeds that floated were eliminated.

Laboratory experiment- Germination tests of the tree species

Clay-loamy soil was transferred into five medium-sized plant bags. The plant bags were set up in the greenhouse, each weighing 3000 g. Various concentrations of crude oil (0, 25, 50, 75, and 100 ml) were artificially added to the soil and thoroughly mixed in. The different concentrations of oil in the soil (0, 25, 50, 75, and 100 ml) indicate the various treatments (uncontaminated, low, average, high, and extremely high contamination, respectively).

With the aid of DTA Series Electronic Scale FED-3000 (Made in China, 2005), 200 g samples of the polluted soil were taken from each treatment (0, 25, 50, 75, and 100 ml), dissolved in 1000 ml measuring cylinders (Technico, England), and then allowed to soak for 72 hours. The aqueous extracts were filtered, and the filtrates were collected and labeled in 500 ml Pyrex conical flasks.

Twenty five petri dishes, each double-layered with Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England), were separated into five groups to simulate the amount of crude oil treatments in the soil (25, 50, 75, and 100) and control, with five replicates each. An identical control experiment was created and repeated five times. To test the germination of the seeds of a few different plant species, ten plant seeds were put in a petri dish and moistened each day at 0700 with filtrates of the appropriate concentrations.

For ten days after sowing, daily counts of germination were made and recorded. Using the formula proposed by Kayode and Oyedeji (2012), the percentage of germination was calculated:

$$\text{Germination test Percentage (Gt \%)} = \frac{\text{Number of seedlings that emerged/dish}}{\text{Total number of seeds sown}} \times 100$$

Greenhouse experiment – Initial growth response of the tree species in soil contaminated with crude oil

Top soil was distributed into fifty (50) medium-sized plant bags (2000 cm3 each) in the greenhouse. They were divided into five groups. Each subgroup was made up of ten planting bags lined up in a row. Different amounts of oil in the soil: 0, 25, 50, 75, and 100 ml were used to contaminate the groups, which represented the control (unpolluted), low, medium, high, and very high pollution rates, respectively.

Two weeks after the pollution (2 WAPo), the plants were watered for two weeks at intervals of 72 hours at 0700, and then three viable seeds were sown in each pot in the Treatments and its Control. Two weeks after planting (2WAP), it was trimmed to one plant per bag.

In order to examine each tree species' first growth response in the crude oil-contaminated soil, measurements of the growth parameters, including plant height and plant girth, were made at 4, 8, 12 and 16 weeks following planting. A Venier Caliper [graduated in millimeter (mm)] was used to measure the seedling girth at the first node above the soil line, and a meter rule [graduated in centimeter (cm)] was used to measure the seedling height between the soil line and the last node toward the upper (aerial) part of the shoot. Each treatment's average height and girth were calculated and documented.

Analysis of the soil samples' physical and chemical characteristics

The various physicochemical parameters of the soil were determined following standard procedures as reported in literatures including bulk density, soil organic carbon, organic matter, soil pH (Ibitoye, 2006), moisture content (Osuji and Onojake, 2004), volume of air in soil, soil water capillarity and porosity

(Akinsanmi, 1975), soil nitrogen, calcium and magnesium (Anderson and Ingram, 1996), available phosphorous (Bray and Kutz, 1945).

RESULTS AND DISCUSSION

The percentage germination of *Leuceana leucocephala* seeds contaminated with crude oil is presented in Figure 1. The percentage germination decreased as the concentration of the crude oil increased. It was also observed that only 75 ml and 100ml concentrations of crude oil significantly inhibited the germination of *Leuceana leucocephala* seeds when compared to the control (0 ml) (Figure 1). The amount of oil present in the soil water extracts determines how much of a decline there is. The results of this study corroborate prior claims made by Kayode et al. (2009), Kayode and Oyedeji (2012), Wang et al. (2017), and Oyedeji and Oyedeji (2022) that crude oil had a negative impact on soil quality, microorganisms, and plants based on the germination rate.

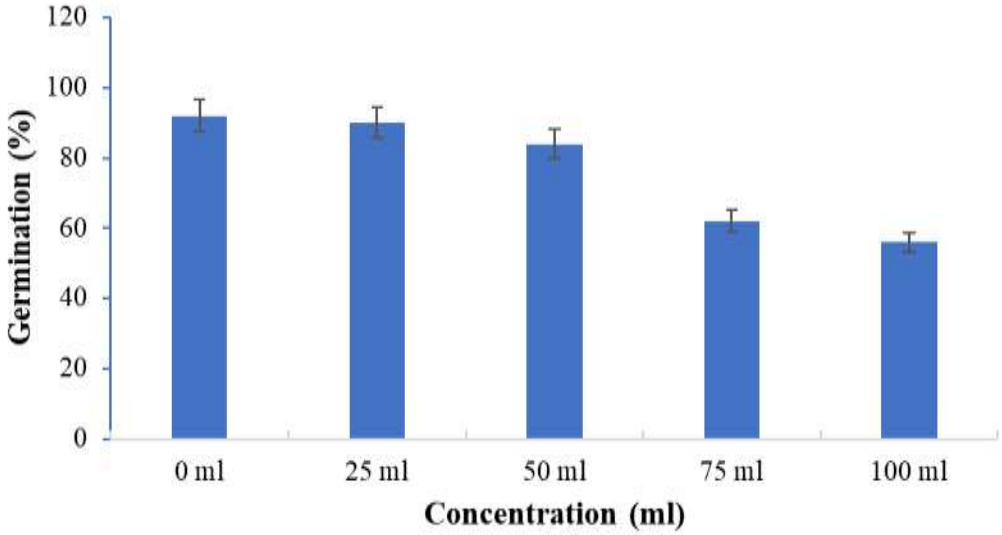

Figure 1: Percentage germination of *Leuceana leucocephala* in crude oil-polluted soil water extracts

The plant height of *L. leucocephala g*rown in crude oil-polluted soil is presented in Table 1. Across the weeks, the plant height reduced as the concentration of crude oil increased. At 8 WAP, the plant height of plants grown in soil treated with 50, 75 and 100 ml of crude oil was significantly ($P \geq 0.05$) lower than the control (0 ml). Furthermore, it was observed that at 12 and 16 WAP, all the concentrations of crude oil in the soil significantly ($P \geq 0.05$) reduced the plant height when compared to the control (0 ml) (Table 1). As a result, as the amount of crude oil in the soil increased, so did the percentage of growth inhibition. The results of this study's analysis of mean height showed a similar pattern to those of Anoliefo and Okoloko's (2000) and Kayode et al.'s (2009a, b), Oyedeji and Oyedeji's (2022) studies, which found that crude oil decreased plants' mean height. The results of this investigation also showed that the heights obtained dropped as the amount of crude oil in the soil increased. This implies that the oil pollution may have prevented the seedlings in the polluted soil from growing.

Table 1: Plant height of *Leuceana leucocephala* in crude oil-polluted soil water extracts

Crude oil concentration (ml)	Time (Weeks After Planting)			
	4	8	12	16
0	13.50±3.14a	33.15±4.80b	51.30±3.15b	73.70±4.40c
25	10.50±3.05a	22.30±4.85ab	38.20±3.01a	58.30±4.75b
50	9.70±4.54a	20.50±5.30a	35.40±3.04a	49.50±4.94ab
75	9.10±1.35a	18.20±4.80a	31.75±3.17a	46.45±3.17ab
100	8.45±3.25a	16.50±2.05a	30.54±4.35a	41.80±6.50a

The plant girth of L. *leucocephala* grown in crude oil-polluted was displayed in Table 2. The plant girth declined at 4, 8, and 12 WAP as the amount of crude oil in the soil become more concentrated. The lowest plant girth was seen at 12 WAP in plants raised on soil contaminated with 75 ml of crude oil. When compared to the control at 16 WAP, the various crude oil concentrations significantly (P 0.05) decreased the plant girth (Table 2). The results of this investigation showed that girth reduced as soil crude oil concentrations increased.

Table 3 shows the effects of various crude oil concentrations on the number of leaves and nodules of L. *leucocephala* at 16 WAP. As the amount of crude oil in the soil increased, the number of leaves and nodules decreased significantly (P 0.05) (Table 3). The decrease in leaf count, nodulation, and girth demonstrated that crude oil has a negative impact on plant growth, especially at higher soil oil concentrations. Therefore, some metabolic processes have been hampered by the presence of crude oil in the contaminated soil. In the majority of studies on plant morphology and physiology, crude oil has been found to alter soil characteristics and hinder plant growth, according to Skrypnik et al. (2021).

Table 2: Mean girth of *Leuceana leucocephala* grown in crude oil-polluted soil

Crude oil concentration (ml)	Time (Weeks After Planting)			
	4	8	12	16
0	0.16±0.02a	0.21±0.01a	0.30±0.05a	0.54±0.03c
25	0.15±0.01a	0.18±0.03a	0.20±0.04a	0.34±0.03b
50	0.14±0.02a	0.17±0.04a	0.16±0.04a	0.33±0.04b
75	0.13±0.02a	0.15±0.06a	0.14±0.05a	0.21±0.03a
100	0.12±0.04a	0.14±0.05a	0.16±0.03a	0.19±0.04a

Table 3: Mean number of leaf and nodules of *Leuceana leucocephala* grown in crude oil-polluted soil at 16 weeks after planting

Crude oil concentration (ml)	Number of leaves	Number of nodules
0	24.00±3.00d	18.00±3.00c
25	16.00±2.00c	10.00±2.00b
50	12.00±1.00bc	10.00±2.00b
75	10.00±2.00ab	6.00±2.00ab
100	6.00±2.00a	2.00±1.00a

Table 4 lists the physiochemical properties of soil that was contaminated with crude oil and treated with L. *leucocephala*. The pH of the polluted soil was 5.22 at 0 ml and 5.03 at 100 ml. However, the control soil was in the 5.72–5.31 range. The pH of soils influences aggregate stability, which in turn affects the movement of air and water in soils, by regulating the amount and type of soil organisms that convert plant wastes into beneficial soil organisms. This finding demonstrates that plants that fix nitrogen, like L. *leucocephala*, were marginally more effective in raising pH. The results of the soil pH measurements in L. *leucocephala* showed that the presence of different oil concentrations significantly changes the soil pH. The pH of the soil is often acidic in samples with high oil content. However, growing nitrogen-fixing plants on such soil, like L. *leucocephala*, had a significant impact on it at 16 WAP. In contrast to the

control, which had organic matter in the range of 3.15 to 2.67%, the concentration of organic matter declined as the crude oil concentration increased, or from 2.06% (0 ml) to 1.71% (100 ml). A typical indicator of soil fertility is the organic matter content of the soil. Another indicator of contamination is organic matter. The carbon mineralizing ability of the soil is strongly correlated with its organic carbon content, which typically results in a decrease in oxygen levels, which in turn impacts microbial metabolism. As a result, the organic matter values have broad implications for mineralization. The decrease in the amount of organic matter in the cleaned-up soils compared to the crude oil-treated soils found in this study demonstrates that L. *leucocephala* possess the metabolic and absorption abilities as well as the transport systems necessary to draw out specific contaminants from the growth matrix.

As the concentration of the crude oil increased, so did the concentration of the fertilizer monitoring parameters (nitrogen, phosphorus, and potassium). The contaminated soil had nitrogen concentrations between 0.50% and 0.35% (0 ml to 100 ml), phosphorous concentrations between 8.65 and 7.65 mg/kg (0 ml to 100 ml), and potassium values between 4.80 and 4.02 mg/kg (0 ml to 100 ml). Basically, the total nitrogen, phosphorus, and potassium concentrations in the remedied soil samples at 16 WAP were different from those in the controls, according to the measurements of the macro elements. This supports the research of Oyedeji et al. (2021) and Oyedeji and Oyedeji (2022), who also found that the majority of macronutrients were changed in the soil at 16 WAP following remediation. According to the research, L. *leucocephala* is capable of keeping the balance between the nitrogen and phosphorus that is accessible in the soil at a level that prevents bioaccumulation or over-reduction that could result in a shortfall. This might be explained by the favorable environment made better by some plants' roots, which improve pollutant availability and mop-up. Some plants have root systems that enable the accumulation of contaminants, according to DalCorso et al. (2019). As a result of these exudates' induction or enhancement of microbial populations, the rhizosphere's ability to degrade organic pollutants is improved. The bacteria executing the degrading process might have used up the additional nitrogen provided to the contaminated soil. Additionally, the immobility (lower availability) of phosphorus contributed to its low level in the remediated soils; it may not have been sufficiently dissolved in the soil to make it available, while the little that was dissolved may have been quickly absorbed by soil microbes.

Table 4: Physiochemical characteristics of crude oil contaminated soil *Leuceana leucocephala* used for remediation

Parameters	Groups	Crude oil concentration (ml)				
		0	25	50	75	100
pH	Contaminated	5.22	5.11	5.07	5.03	5.03
	Control	5.72	5.54	5.46	5.36	5.31
Organic matter, %	Contaminated	2.06	1.96	1.85	1.71	1.71
	Control	3.15	3.00	2.85	2.78	2.67
% N	Contaminated	0.50	0.48	0.45	0.40	0.35
	Control	0.91	0.88	0.81	0.75	0.48
P, mg/kg	Contaminated	8.65	8.20	8.05	7.85	7.65
	Control	15.30	14.52	13.95	13.50	11.85
K, mg/kg	Contaminated	4.80	4.35	4.18	4.05	4.02
	Control	11.50	10.20	10.20	9.50	9.35
Na, mg/kg	Contaminated	2.35	2.25	2.12	2.12	2.12
	Control	5.60	5.20	4.85	7.75	8.45
Ca, mg/kg	Contaminated	28.05	27.25	25.30	23.80	21.80
	Control	46.50	45.10	45.20	43.50	40.50
Mg, mg/kg	Contaminated	2.25	2.10	2.05	2.03	2.03
	Control	2.74	2.60	2.60	2.36	2.16

The concentration of sodium ranged between 2.35 mg/kg (0 ml) – 2.12 mg/kg (100ml), calcium ranged between 28.05 mg/kg (0 ml) – 21.80 mg/kg (100ml) and magnesium ranged between 2.25 mg/kg (0ml) – 2.03 mg/kg (100ml). According to the findings of the soil studies, calcium ion had the highest concentration of observed exchangeable cations in the soils that had been contaminated by crude oil, followed by sodium ion, and magnesium had the lowest concentration.

The outcome showed that L. *leucocephala* was successful in lowering the cation concentrations, with calcium having the most impact. Plant nutrients that reside as ions in the rhizosphere are transported by soil particles. With an increase in crude oil pollution, the concentrations of the exchangeable cations (calcium, magnesium, and sodium) increased. The studies by Oyedeji et al. (2021) and Oyedeji and Oyedeji (2022) revealed similar trends.

The physical characteristics of the soil utilized in the experiment, which was both unpolluted and polluted with crude oil, are shown in Table 5. Bulk densities of 5.80, 6.40, 6.40, 6.8 and 7.70 g/cm3 were recorded for the treatments with soil that had been exposed to 25 ml, 50 ml, 75 ml, and 100 ml of crude oil, respectively. The soil samples that had been contaminated with crude oil had less soil moisture, especially the 100 ml sample. Similar to how it impacts soil air, the amount of crude oil was detected in soil samples of 0, 25, 50, 75, and 100 ml at 72.50, 38.60, 30.50, 40.40, and 43.60%, respectively. The soil's ability to contain water was also diminished due to crude oil pollution.

Table 5: Physical properties of unpolluted and crude oil-polluted soil

Treatment (ml)	Bulk Density(g/cm^3)	Moisture content (%)	Soil air (%)	Water Holding capacity (ml)	Soil porosity(ml)
0	5.8	72	72.5	58.4	86.4
25	6.4	44.5	38.6	50	81.5
50	6.4	40	30.5	34.5	60.4
75	6.8	28.5	40.4	24.1	48.3
100	7.7	18.2	43.6	13.7	32.8

It has been shown that soil's physical characteristics are affected by the presence of crude oil. The bulk density, moisture content, soil air, water holding capacity, and porosity of the crude oil-polluted soil decreased as the crude oil concentration rose. This backs up Ahmadi et al.'s report from 2021. The effective cation exchange capacity is changed by crude oil, which therefore affects base saturation.

CONCLUSION

Crude oil in soil affected the soil physicochemical characteristics like nitrogen, phosphorous, calcium, sodium, magnesium, potassium and organic matter. *L. leucocephala* showed it could thrive in crude oil with regard to germination, height, leaf number and nodulation, which tend to decrease with the concentration of the crude increase. In light of this, L. *leucocephala* is a suitable candidate plant species for the phytoremediation and re-vegetation of crude oil-contaminates soil.

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