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Phytoremediation Potential of *Senna fistula* L. in the Decontamination of Crude Oil-Polluted Soil

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ABSTRACT: Crude oil contaminated soil could be treated using physicochemical, thermal or biological managements. However, the objective of this paper is to assess the phytoremediation potential of *Senna fistula* L in the decontamination of crude oil-polluted soil in a greenhouse located at Wilberforce Island, Niger Delta Area, Nigeria using appropriate standard methods. This study examined the survivability of *Senna fistula* in soil contaminated with crude oil with a view to ascertain its potential in bioremediation. Experiments to assess the germination and growth rates of *S. fistula* were conducted in a greenhouse, using soil contaminated with varying concentrations of crude oil (25%, 50%, 75% and 100%). Germination rate and growth parameters (number of leaves, nodulation, plant girth and height) were ascertained in accordance with scientific methods. Equally, the physicochemical properties of the soil (organic matter, pH, calcium, magnesium, sodium, phosphorous, potassium and nitrogen) were determined in accordance with scientific methods. Results revealed that at 0% 25%, 50%, 75% and 100% crude oil in soil the corresponding germination rates for *S. fistula* were 26%, 22%, 22%, 15% and 14% in that order. The mean heights of the plant 16 weeks after planting, were 52.30cm, 34.50cm, 26.50cm, 25.40cm and 20.80cm; mean girths 0.40mm, 0.35mm, 0.35mm, 0.25mm and 0.20mm; number of nodules 8, 2, 0, 0 and 0 and number of leaves 13.00, 8.00, 8.00, 7.00 and 4.00, 0% for 0%, 25%, 50%, 75% and 100% crude oil concentrations, in that order. Organic matter in the contaminated soil was in the range of 4.15%-4.73%; pH, 3.34-4.25; N, 0.30-0.41%; P, 6.15- 6.38 mg/kg; Na, 2.05-2.81 mg/kg; Ca, 12.85-14.50 mg/kg, and Mg, 1.45-1.91 mg/kg. An inverse proportionality was observed between measured parameters and crude oil concentrations. However, the germination efficiency and the relative growth rate of the plant evinced survivability under crude oil chemical stress and its fittingness for use in soil remediation in the Niger Delta.

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Environmental pollution diminishes the development pace of trees and bushes, and may try and bring about the debility of forest stands, owing to soil pollution, which has a negative effect on the development of root systems (Oyedeji, 2016). Soil pollution arising from crude oil has a number of adversative effects on ecosystems affecting from the smallest of life forms (microorganisms) to humans, even though they be veiled and slow to manifest (Wegwu *et al.*, 2011; Ordinioha and Brisibe, 2013; Onwuna *et al.*, 2022a, Aigberua *et al.*, 2016). The harmful upshots of crude oil pollution occurs when its presence in any environmental matrix, is in high amount, sufficient to be of risk to biota and soil health. Traditional fishing in the Niger Delta can no longer be a means of sustenance, as such, rural dwellers are either turning to crop growing or supplement fishing with farming. The productive capacity of arable land in the Niger is constantly under the threat of crude oil pollution, with reports suggesting that it's fast losing its productive capacity (Oyedeji *et al.*, 2012; Oyedeji, *et al.*, 2015). Hectares of farmland in the Niger Delta lie abandoned

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because their productive quality has been severely degraded (UNEP, 2011). Although crude oil in the Niger Delta has been blamed on the criminal negligence of both local and international oil companies as well as the rising activities of crude oil bunkering and artisanal crude oil operation (Yabrade and Tanee, Onwuna *et al*., 2022a, Onwuna *et al*., 2022b). The only hope for ecosystem restoration and guarantee of local food supply in the Niger Delta is through cleanup of contaminated matrices by biological and chemical approach (UNEP, 2011; Zabbey *et al*., 2017). Bioremediation techniques adopted to exclude pollutants from the soil should include the use of plants that survive harsh chemical stress and accumulate the pollutants (Alkorta and Garbisu, 2001; Oyedeji 2022). Stanisławska-Glubiak *et al*. (2012), Karczewska *et al*. (2013) and Al-Ateeqi (2014) itemized the benefits of using native plants for phytoextraction and phytostabilisation, particular those that have a fast growth rate, well-developed root system, large in size and are tolerant to pollutants. In this regard, plants that grow well in crude oil-contaminated soil tend to be used for phytoremediation (Bamidele and Agbogidi, 2006). Therefore, before plants can be developed for possible utilization in the remediation of toxicants, they should demonstrate tolerable growth in the presence of the pollutant. Because of the role of rhizosphere microflora in assisting plants in the process of pollutant removal and degradation (Germida *et al*., 2010; Deka *et al*., 2009; Al-Ateeqi, 2014), leguminous plants have been recommended for use in phytoremediation. *Senna fistula* is a legume known as the golden shower tree that belongs to the family fabaceae. This study seeks to assess the phytoremediation potential of *S. fistula* in the decontamination of crude oil-polluted soil.

MATERIALS AND METHODS

Study Site: This study was carried out in a greenhouse located in the Faculty of Science, Niger Delta University, Nigeria.

Sample Collection: *Senna fistula* seeds was sourced from the National Centre for Genetic and Biotechnology (NAGRAB), Ibadan, Nigeria. Clay-loamy from the top 0-10 cm depth was collected from the Experimental Farm of the Niger Delta University (NDU), Nigeria. Crude oil, Bonny Light grade, was provided by Shell Petroleum Development Company from its Oporoma Flow Station.

Germination test: Viable *P. osun* seeds selected by the floating method (Anoliefo and Vwioko, 1995), were planted in five medium-sized plant bags (2000cm³) holding 3000 g of soil. Five treatments were set (Treatment A-0%, Treatment B-25%, Treatment C-50%, Treatment D-75% and Treatment E-100%) with 0 ml, 25 ml, 50 ml, 75 ml or 100 ml of crude oil, where the 0 ml uncontaminated treatment served as the control.

Aqueous soil extract was obtained from 200 g of soil spiked with either 25 ml, 50 ml, 75 ml or 100 ml of crude oil and the control in distilled water (1000 ml) after 72 hours. Aqueous extracts of the soil samples were used to wet the seeds in the germination test, as per the methods Kayode and Oyedeji (2012). The test was set in five replicates with ten seeds per petri dish. The seeds were wetted daily 0700 for 10 days. Germination counts was taken daily and the percentage germination was determined on the 10th day using the formula as in equation 1:

$$\%Gt = \frac{\text{No seedlings emerged/dish}}{\text{Total number of seeds sown}} * 100 \quad (1)$$

Where Gt = Germination test Percentage (Gt %); No Seeding Emerge = number of seeding that emerged

Growth response test: The growth response test was conducted as per the method of Kayode and Oyedeji (2012) using 3 viable seeds per bag. The number of germinated plants per bag was pruned to allow one per bag 2 weeks after planting (WAP). Growth parameters (plant height, number of leaves, nodulation and plant girth) were measured 2 WAP and then 16 WAP. The relative growth rate (RGR) and percentage growth suppression (%GS) were estimated from mean heights.

Analysis of the physical properties of the soil samples: Soil physicochemical parameters were determined are moisture content as described by Osuji and Onojake (2004); volume of air in soil, soil water capillarity and porosity as described by Akinsanmi (1975); soil nitrogen, calcium and magnesium as described by Anderson and Ingram, (1996), phosphorous determined colorimetrically as described by Bray and Kurtz (1945); while soil organic matter, pH and bulk density were determined as described by Ibitoye (2006).

RESULTS AND DISCUSSION

Figure 1 shows the germination rate of *S. fistula* contaminated. At varying concentrations of 0%, 25%, 50%, 75% and 100% of crude oil, the percentage germination of *S. fistula* was 26% (21 COV), 22% (17 COV), 22% (17 COV), 15% (9 COV) and 14% (6 COV) respectively. *S. fistula* in crude oil-polluted soil showed a decrease germination rate as concentration of oil in the soil increases. The observed germination rate in this study is in congruence the works of Osuji *et al.* (2005); Oyedeji and Oyedeji (2012) who reported that crude oil has an adverse effect on soil biotic and abiotic properties, depending on the concentration of the crude oil. Adeyemi and Adeyemi (2020) attributed the deleterious effects crude oil, such as plant germination, to be as a result of the cytotoxicity of crude oil constituents.

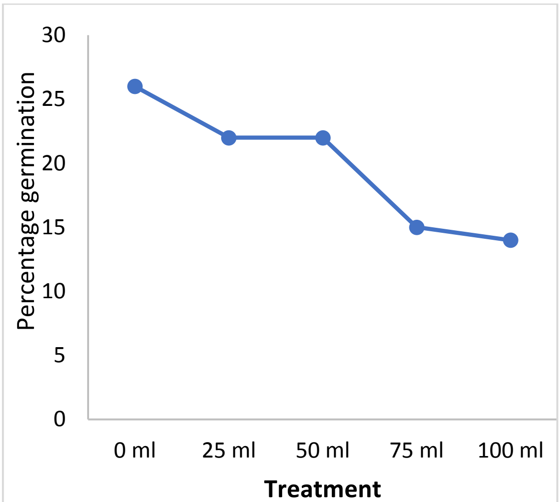

Fig 1: Percentage germination of *S. fistula* in crude oil-polluted soil water extracts

Table 1 shows the mean height of *S. fistula*. The mean heights of the plant 2 WAP were 0.85±1.20cm, 2.65±1.55cm, 2.80±1.80cm, 1.82±1.45cm and 1.78±2.30cm for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments correspondingly. The mean heights 16 WAP were 52.30±1.72 cm, 34.50±1.54cm, 26.50±1.80cm, 25.40±1.44cm and 20.80±2.00cm for the 0%, 25%, 50%, 75% and 100% crude oil-spiked soil treatments in that order. The result revealed that the mean heights oof *S. fistula* decreased with an increase in the concentration of crude oil in the soil. The RGR ranged from 0.29-0.19. The percentage growth suppression ranged from 34-60.2%. Thus, the percentage growth suppression increased with an increase in the concentration of the crude oil in the soil. This agrees with report of Adeyemi and Adeyemi (2020) Oyedeji and Oyedeji (2022) where it was established that crude oil pollution suppressed the growth of plants. Table 2 shows the mean girths of *S. fistula* with values of 0.12 mm, 0.12 mm, 0.11 mm, 0.10 mm and 0.10 mm, for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments correspondingly 12 WAP, whereas 16 WAP the values increased to 0.40±0.03mm, 0.35±0.04mm, 0.35±0.03mm, 0.25±0.03mm and 0.20±0.04mm, for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments correspondingly. The findings of this study showed a decrease in girth with an increase in the concentrations of crude oil in the soil. Adeyemi and Adeyemi (2020) similarly reported decrease in plant girth with crude oil concentration in their study in which the leguminous plant *Phaseolus Vulgaris* L. The reduction in girth like height, is likely as a result of reduced availability of soil nutrient and oxidative stress, as alluded to by Okon and Udofot (2012) Adeyemi and Adeyemi (2020).

Table 1: Mean plant heights of *S. fistula*

Time (WAP)	Plant height (cm)/Crude oil concentration				
	0%	25%	50%	75%	100%
2	0.85±1.20	2.65±1.55	2.80±1.80	1.82±1.45	1.78±2.30
4	5.50±2.20	7.20±1.46	6.70±1.35	5.75±1.60	3.50±2.60
6	15.20±2.50	18.70±.175	11.70±1.65	13.70±1.48	6.70±2.05
8	23.50±1.85	20.20±1.54	15.40±1.40	14.10±1.32	11.40±1.80
10	23.70±1.72	26.50±1.26	18.60±1.54	16.70±1.58	15.50±1.92
12	35.20±2.10	30.10±1.70	24.20±1.66	19.15±1.48	17.80±2.15
14	40.70±1.65	35.40±1.67	25.80±1.57	23.15±1.64	18.80±1.88
16	52.30±1.72	34.50±1.54	26.50±1.80	25.40±1.44	20.80±2.00
∑X±SD	**196.95±14.94**	**175.25±10.90**	**131.70±12.77**	**119.77±11.99**	**96.28±16.70**
$\Delta H = H_F - H_I$	**51.45±0.52**	**31.85±0.01**	**23.70±0.00**	**23.40±0.01**	**19.02±0.30**
RGR	**0.29**	**0.18**	**0.16**	**0.19**	**0.18**
GS	**0.00**	**0.340**	**0.493**	**0.514**	**0.602**
%GS	**0.00**	**34.00**	**49.30**	**51.40**	**60.20**

Where RGR = Relative growth rate; GS = growth suppression; H_I = Initial Height; H_F = Final Height; ΔH = Change in height; $\bar{X}$ = Mean; (±) = Standard deviation

The number of leaves 13.00, 8.00, 8.00, 7.00 and 4.00 for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments, correspondingly (Table 3). The number of nodules were 8, 2, 0, 0 and 0, for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments, correspondingly (Table 3). One obvious indication of decrease in the biological performance of in presence of a pollutant is manifested in the form of reduction leaf numbers, and for legumes lack of nodules would stand out. The decrease in the number of leaves and reduced nodulation, as it is, at higher measure of the oil in soil clearly suggest that the pollutant suppressed the growth of the plants. Reduction in leaf number and nodulation in the instance of crude oil in soil, is likely to be as a result impaired metabolic processes in plant grown in polluted soil.

Table 2: Mean girth of *S. fistula*

Experimental Time (WAP)	Plant girth (mm)/Crude oil concentration				
	0%	25%	50%	75%	100%
2	0.12±0.02	0.12±0.03	0.11±0.01	0.10±0.02	0.10±0.02
4	0.16±0.02	0.12±0.02	0.12±0.03	0.11±0.03	0.10±0.02
6	0.16±0.02	0.15±0.03	0.12±0.02	0.12±0.03	0.11±0.03
8	0.16±0.02	0.15±0.03	0.12±0.02	0.12±0.03	0.11±0.03
10	0.24±0.03	0.20±0.04	0.15±0.03	0.12±0.02	0.12±0.04
12	0.31±0.04	0.25±0.03	0.25±0.03	0.15±0.04	0.15±0.03
14	0.37±0.03	0.30±0.03	0.28±0.03	0.20±0.04	0.15±0.03
16	0.40±0.03	0.35±0.04	0.35±0.03	0.25±0.03	0.20±0.04
$\Delta G = G_F - G_I$	**0.28±0.01**	**0.23±0.01**	**0.24±0.02**	**0.15±0.01**	**0.10±0.02**
$\sum X \pm SD$	**1.92±0.21**	**1.64±0.25**	**1.50±0.20**	**1.17±0.24**	**1.04±0.24**

Table 3: Mean number of leaf and nodules of *S. fistula*

Mean number	**0%**	**25%**	**50%**	**75%**	**100%**	**Mean**	**Variance**	**SD**
Leaf	13.00	8.00	8.00	7.00	4.00	8.00	10.50	3.24
Nodules	8.00	2.00	0.00	0.00	0.00	5.00	18.00	4.24

Table 4 shows the physiochemical characteristics of the soil. Organic matter in the soil was in the range of 4.15%-4.73%; pH, 3.34-4.25; N, 0.30-0.41%; P, 6.15-6.38 mg/kg; Na, 2.05-2.81 mg/kg; Ca, 12.85-14.50 mg/kg, and Mg, 1.45-1.91 mg/kg. The pH of soils was within the acidic range. Soil pH helps shapes the microbial communities in soil and affects the movement of air and water in soils. The plant, *S. fistula* caused slightly increase soil pH, and could prove useful for the improvement of soil properties where reduction in pH is required. Like pH, the organic matter concentration dropped as the concentration of the crude oil increased i.e. 1.32% (0 ml) - 1.08% (100ml), whereas the control had organic matter in the range of 1.65 – 1.55%. Although, organic matter content of soils is an index of soil fertility. it is also a pollution indicator. Soil organic matter influences soil microbial activities and it is as well influenced by microbes through organic matter decomposition. Thus, the reduction of the level of organic matter in the polluted soils may be as a result of the effect of crude oil on microorganism, which might reduce the population of beneficial degraders. The concentrations of all mineral element monitored also decreased as the concentration of the crude oil increased, albeit marginally. The exchangeable cations in the soil were in the order Ca>K>Na>Mg, with the highest concentrations detected in uncontaminated treatment. However, crude oil in the soil makes nutrient unavailable to plant (Wang *et al*., 2013; John *et al*., 2016).

Table 4: Physiochemical characteristics of crude oil-contaminated soil that *S. fistula*

Parameters	Crude oil concentration				
	0%	25%	50%	75%	100%
pH	4.25	4.05	4.01	3.81	3.34
Organic matter (%)	1.32	1.24	1.14	1.08	1.08
N (%)	0.41	0.37	0.32	0.30	0.30
P (mg/kg)	6.38	6.34	6.24	6.15	6.15
K (mg/kg)	2.72	2.61	2.55	2.50	2.50
Na (mg/kg)	2.81	2.75	2.55	2.25	2.05
Ca (mg/kg)	14.50	14.10	13.85	12.85	12.85
Mg (mg/kg)	1.91	1.80	1.60	1.45	1.45

Table 5 shows the effect of the physical properties of unpolluted and crude oil-polluted soil used in the experiment. Bulk densities of 5.80, 6.40, 6.40, 6.8 and 7.70 g/cm^3 were observed in the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments, correspondingly. Soil moisture content reduced in the crude oil-polluted soil samples, particularly in the 100 ml crude oil-contaminated soil. Similarly, the presence of crude oil in the soil samples affects the soil air, 72.50, 38.60, 30.50, 40.40 and 43.60 % were observed for the 0%, 25%, 50%, 75% and 100% crude oil-polluted soil treatments, correspondingly. Water holding capacity was also reduced in the crude oil-polluted soil. The presence of crude oil in soil affects the physical properties of soil, in a way that as the percentage of the crude oil increased, the soil bulk density, moisture content, soil air, water holding capacity and porosity reduced in the crude oil-polluted soil. This is in consonant with the previous investigation by Adeyemi and Adeyemi (2020) that reported the upshot of crude oil on *Phaseolus Vulgaris* L to include reduced availability of water and in soil.

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

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

Conclusion: Crude oil in soil reduces soil physicochemical characteristics such as nitrogen, phosphorous, calcium, sodium, magnesium, and potassium organic matter. *S. fistula* showed tolerance to crude oil pollution in its germination rate, height, leaf number and nodulation, though these parameters decreased as the concentration of the crude increased. Therefore, *S. fistula* has the potential for phytoremediation of soil contaminated with crude oil.

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