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Full length Research Paper

Bioremediation of oil polluted soil: Effect on hill bamboo (*Drepanostachyum falcatum*) plant emergence and height

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This study presents the bioremediation effect on the performance of hill bamboo *Drepanostachyum falcatum* on different soil combinations affected by crude oil pollution. Bioremediation was applied into the soil combination substrates by adding biodegrading bacteria using *Pseudomonas fluorescen* and *Bacillus subtilis*. Six treatments made with combinations of soil, bacteria, fertilizer and crude oil were used for this study. Increase in the plant height was observed as a result of treatment combination of soil, crude oil, bacteria and fertilizer. It also increased the proportion of plant emergence. The results were not so significant with other crude oil pollution treatments. These results open the door for more research to investigate the scalability of this bioremediation and also the possibility to have the same bioremediation effect against other type of pollution.

Key words: Soil microorganisms, bioremediation, pollution, plant growth, hill bamboo.

INTRODUCTION

Bioremediation is the term applied to the technologies that accelerates natural processes for degrading and /or harmful toxic chemicals in soil, groundwater and wastewater. Bioremediation is defined as "the use of living organisms to reduce or eliminate environmental hazards resulting from accumulations of toxic chemicals and other hazardous wastes" (Gibson and Saylor, 1992). This definition is accepted by the American Academy of Microbiology.

Crude oil pollution has been reported to have deleterious effects on plant germination and seedling growth (Kyung-Hwa et al., 2004). Bioassays such as measurements of seed germination and early seedling growth have been used to monitor treatment effects of oil-contaminated sites (Sverdrup et al., 2003). Dorn and Salanitro (2000) found that seed germination and plant growth using corn, wheat, and oats differed from different soils and oil combinations before, during, and after bioremediation. Sayles et al. (1999) showed that oil contaminated soil treated with aerobic biodegradation was less toxic to lettuce and oat root elongation. Hanson et al. (1997) reported that Acinetobacter sp. A3-treated soil permitted better germination and growth of mung beans, as evidenced by better plant length, weight, and leaf chlorophyll content.

The adverse effects of oil pollution on economic plants

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Table 1. Design of treatment combinations.

Treatment (no.)	Treatment combinations			
	Soil	Crude oil	Microbes	Fertilizer
1	✔	-	-	-
2	✔	✔	-	-
3	✔	✔	✔	-
4	✔	✔		✔
5	✔	✔	✔	✔
6	✔	-	-	✔

have been reported (Odu et al., 1981; Isirimah et al., 1989; Amedi et al., 1993; Anoliefo and Okoloko, 2000; Ogbonna et al, 2007; Iyagba, 2014). At high concentrations of oil in soil, most plant species suffered serious depression in growth (Udo and Fayami, 1975; Amakiri and Onofeghara, 1984). This condition has been attributed to poor soil conditions, dehydration and impaired nutrient uptake by the roots, created by the presence of crude oil (Anoliefo et al., 2003).

Microbial degradation of oil products is a principal process in the elimination of petroleum from the environment (Zobell et al., 1964). Microbes such as bacteria, existing in the ecosystem can affect hydrocarbons present in oil components by degrading it further. Hence, those microorganisms have the potential to provide solution for oil pollution, especially as regard to plant growth. Bioremediation is concerned with biological restoration and rehabilitation of contaminated sites (Mudasir et al., 2016). There are basically two main approaches to oil bioremediation:

Bioaugmentation: The addition of oil-degrading bacteria to supplement the existing microbial population.

Biostimulation: The addition of nutrients or growth enhancing co-substrates and/or improvement in habitat quality to stimulate growth of indigenous bacteria.

Considering the increase in oil pollution damages on the ecosystems around the world, more emphasis should be put on this natural possible solution. Studies published in this area yield lack effective data support on bioremediation effects and growth of danda crops in wasteland. The present study aims to demonstrate the growth of hill bamboo (*Drepanostachyum falcatum*) as a result of bioremediation, in association with the soil physico-chemical property. Hill bamboo, a danda crop, is found in the Uttarakhand state of India and is widely consumed. The growth of hill bamboo will thus be evaluated on basis of availability of major nutrients and the plant growth.

MATERIALS AND METHODS

Untreated topsoil (0 to 15cm) with similar physico-chemical characteristics of wasteland soil, having no fertilizer and crude oil treatment, was collected from the Forest Research Institute, Dehradun, India with similar physico-chemical properties of wasteland soil that has no previous history of fertilizer application nor has it received crude oil spill. The quantity of crude oil was 100 ml obtained from Indian Institute of Petroleum, Dehradun. The treatment combination pots contained 2 kg of soil each. The ratio of fertilizer N.P.K was 15:15:15. Biodegrading bacteria *Pseudomonas fluorescen* and *Bacillus subtilis* were obtained from Institute of Microbial Technology, Chandigarh, India. Viable seeds of Hill bamboo (*Drepanostachyum falcatum*) obtained from seed testing laboratory, Forest Research Institute, Dehradun, India.

Preparation of different treatment combinations of soil

To get 4% crude oil pollution level, 100 ml of oil was mixed in the soil treatment pots with 2 kg of soil in each pot. After two days, N.P.K fertilizer was added in the ratio of 15:15:15 at the rate of 200 kg/ha. In addition to the fertilizer, biodegrading bacteria as *P. fluorescen* and *B. subtilis* at the rate of 10^6cells/ml were added to the top soil treatment pots.

Based on the above arrangements, six treatment combinations were designed for further experimentation. This design was planned in a manner to determine the physico-chemical properties of treatment combinations pre-application, during application process and post application process in the experiment. The treatment combinations can be depicted in Table 1.

Fifteen viable seeds of Hill bamboo (*Drepanostachyum falcatum*) were planted in each treatment pot, seven days after the earlier mentioned treatment combinations. Water was later filled in the pots with seedlings of *D. falcatum*. The percent of crop emergence and plant height characteristics were evaluated after two and ten weeks after planting (WAP).

Soil chemical property determination methods

Physico-chemical properties of soil were examined in pre-application, during application process and post application of the treatments. Different soil properties were determined by different methods. The wet dichromate oxidation method of Walkley and Black (Nelson and Sommer, 1982) to find total organic carbon; semi macro Kjedahl digestion procedure for total nitrogen content; the Wetenabe and Olsen acid modification of the molybdate blue method (Olsen and Sommer., 1982) to find total phosphorus content; and p-xylene extracted at 415nm spectrophotometrically (Aneilofo et al., 2003) for determination of total petroleum hydrocarbon.

RESULTS

Physico-chemical properties of soil

Soil pH and organic matter strongly affect soil functions

Table 2. Soil physical and chemical properties during pre-application of treatment.

Soil properties	Before experiment					
	T1	**T2**	**T3**	**T4**	**T5**	**T7**
Soil pH	7.40	7.43	7.46	7.47	7.46	7.45
Organic Carbon (O.C) (%)	3.90	6.15	6.54	6.46	5.76	6.65
Total Nitrogen (TN) (%)	0.371	0.560	0.595	0.676	0.678	0.681
Available Phosphorus (P) (mg/kg^{-1}) (%)	72	62	60	58	55	45
Potassium (K+) (%)	19.2	13.5	13.5	13.5	14.5	18
Total Hydrocarbon (mg/kg^{-1})	0.65	164.40	129.45	94.45	80.35	0.66

Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer.

Table 3. Soil physical and chemical properties during the experimental process.

Soil Properties	During experiment					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Soil pH	7.66	8.14	8.05	8.07	8.08	8.06
Organic carbon (O.C) (%)	2.12	3.40	3.90	3.93	4.10	2.42
Total Nitrogen (TN) (%)	0.202	0.324	0.371	0.374	0.390	0.230
Available Phosphorus (P) (mg/kg^{-1}) (%)	24.4	25.2	16	14.4	16.4	22
Potassium (K+) (%)	22.4	21	20	20	21	21.5
Total Hydrocarbon (mg/kg^{-1})	0.65	163.50	129.45	94.43	80.30	0.65

Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer.

Table 4. Soil physical and chemical properties post application of treatment.

Soil properties	After experiment					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Soil pH	7.62	8.00	8.06	8.22	8.14	8.06
Organic Carbon (O.C) (%)	3.62	5.07	6.23	5.60	5.30	4.07
Total Nitrogen (TN) (%)	0.345	0.461	0.567	0.510	0.482	0.388
Available Phosphorus (P) (mg/kg^{-1}) (%)	30.4	26.4	24	24.8	32	24
Potassium (K+) (%)	21	20.5	20.5	20.5	22.5	22
Total Hydrocarbon (mg/kg^{-1})	0.66	164.50	130.40	94.40	79.85	0.67

Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer.

and plant nutrient availability (McCauley et al., 2009). The pH of the soil determines the type of microorganisms that can participate in hydrocarbon degradation (Bassert and Booth, 1984). The pH of the soil samples is depicted in Tables 2, 3 and 4 for the pre-application, during application process and post application of the treatment respectively. The treatment results indicate negligible effect on the soil's pH, which remained neutral. The mineralization of hydrocarbon proceeds most rapidly at pH levels between 7.0 and 8.5 (Dibble and Bartha, 1979) and limited tolerance of bacteria for acidic conditions, unlike fungi being more tolerant under similar conditions (Alexander et al., 1978). Thus, it can be stated that the mineralization of crude oil is affected by pH. Njoku and Oboh (2012) evaluated the pH, organic matter, microbial population, total hydrocarbon of soil and comparative effects of *Abelmoshus esculetus* (Okro) and *Corchorus olitorius* on soil contaminated with petroleum products.

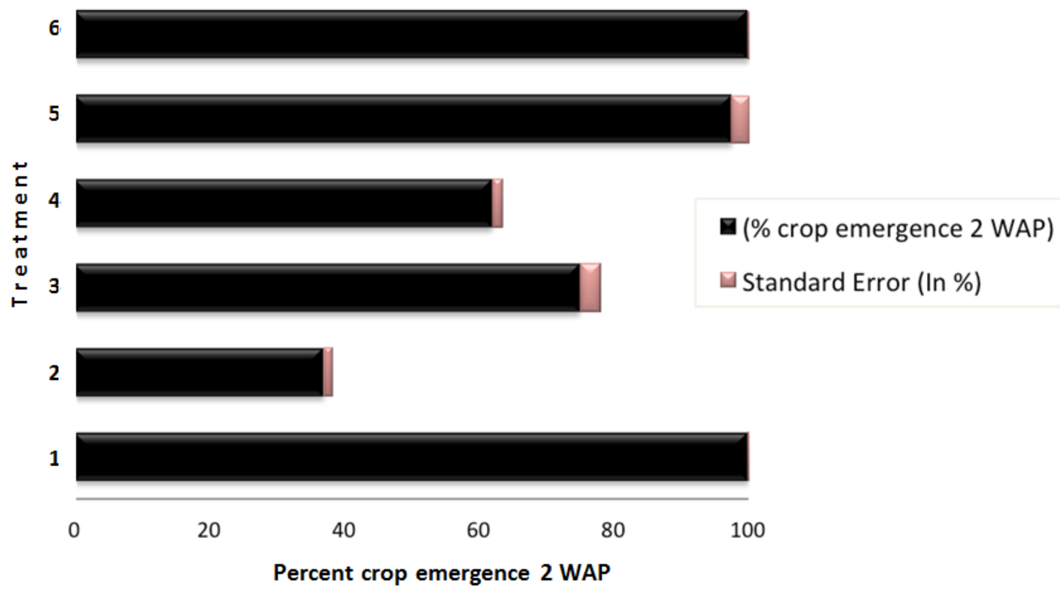

Figure 1. Effect of bioremediation on percent crop emergence of (Hill Bamboo) two weeks after planting in crude oil polluted soil (Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer).

Significant reduction in the total organic carbon levels, due to the introduction of microbes was also noticed. This can be supported by the fact that large amounts of organic carbon in absence of nitrogen led to buildup of microbial biomass. Microbial population growth reduces as a result of sudden increase in the number of microbes which also depletes the existing supplies. Hence, microbial population can keep on growing only if sufficient nutrient supply is continued (Hoff et al., 1992).

As compared with the control condition, a decrease in the levels of nitrogen was observed during incubation. The after effect of biodegradation activities was seen in decreased level of nutrients. The table 2 depicts the result of soil properties-pre-application, during application process and post application of treatment and harvesting. The results indicate markable increase in the organic carbon levels from 3.90 to 6.65%, also an increase in total nitrogen was noted from 0.37 to 0.68%, while total phosphorous showed reduced levels from 72 to 45%.

Hydrocarbon degradation

The treatment combination (6) of soil and fertilizer showed reduced level of petroleum hydrocarbon in the soil as compared to the control. The effect of degradation reached 72% of the total petroleum existing in the soil which is due to the fact that the fertilizer increased the activities of the added bacteria. The various treatment combinations – 3, 4 and 5 showed significant reduction ($p<0.05$) as compared with treatment 2. The treatment combination 5 reflected the highest level of degradation of crude oil. The microorganisms synthesize and utilize the petroleum hydrocarbon as sole source of carbon and energy (Amadi and Bari, 1992). Microorganisms that can convert toxic organic compounds to harmless products, are used as remediation of contaminants such as petroleum hydrocarbons (Lovley and Lloyd, 2000). Olusola and Anslem (2010) reported bioremediation of crude oil polluted soil with *Pleurotus pulmonarius* and *Glomus mosseae* on *Amaranthus hybridus* plant.

Bioremediation effect on percent crop emergence

The results imply that not only the emergence of crop was delayed by 2 to 4 days but also significant reduction in the percentage of crop emergence was seen as a result of 5% level of crude oil addition in the soil. Microbes and fertilizer added separately, or together showed improvement in the crop emergence within 1 to 2 days. The percent of crop emergence dropped from 100% from the control condition (treatment 1) to 37% (Figure 1) when planted on treatment 2 combination of soil and crude oil. Nwogu et al. (2015) demonstrated that *Capra aegagrus hircus* (Goat) manure is a good biostimulant. The crop emergence improved to 75 and 62%, the hill bamboo was planted on polluted soil enriched with bacteria (treatment combination 3) or with fertilizer (treatment combination 4) respectively. The proportion of emergence of the hill bamboo reached 97%, when both bacteria and fertilizer are added to the polluted

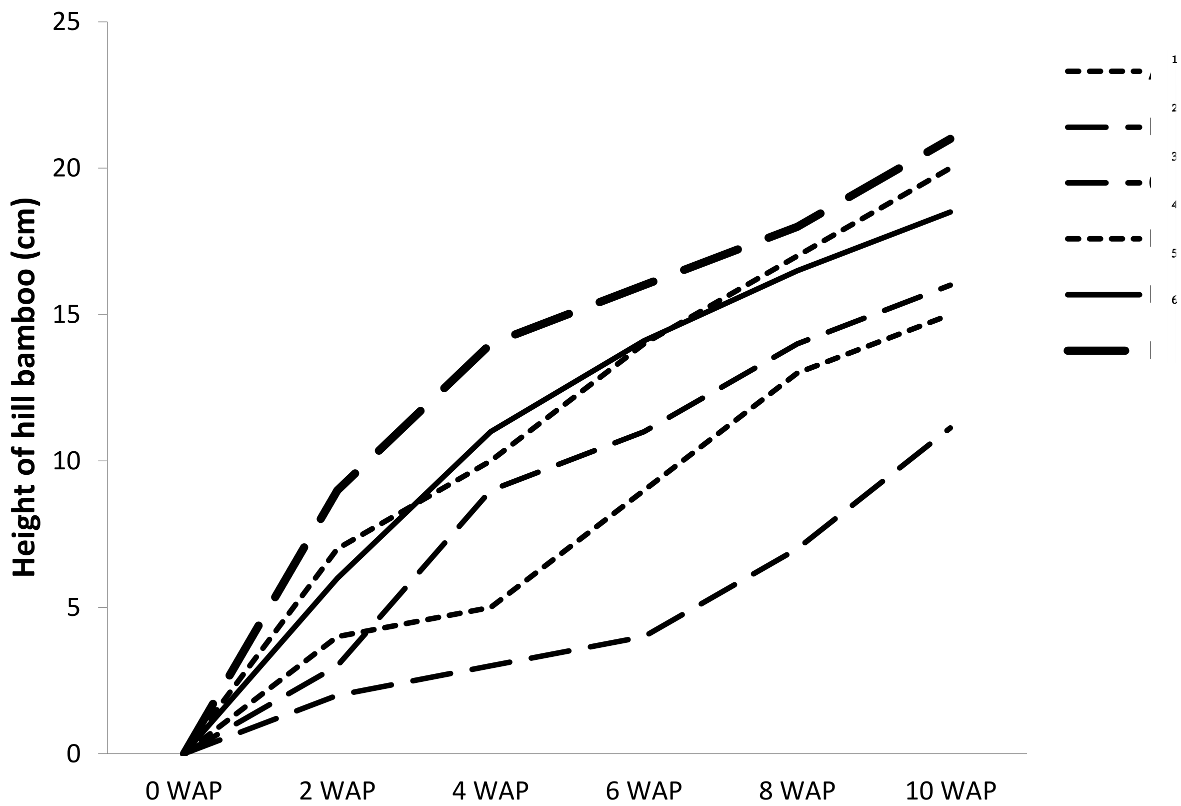

Figure 2. Bioremediation effect on plant height of hill bamboo planted in crude oil polluted soil (Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer).

soil (treatment 5). The pollution in the soil clearly affected negatively the emergence of hill bamboo. A negative effect, due to the addition of bacteria and fertilizer, alone or in association, seems to be correct.

The application of bacteria and fertilizer on non-polluted soil allow 100% emergence of hill bamboo plants, similar to the control. This shows that the bacteria and the fertilizer have no negative effect on the emergence of hill bamboo plants.

Effect of bioremediation on plant height

The stunted growth of plant as caused by crude oil pollution was eliminated significantly, as a result of the application of microbes and mixture of microbes and fertilizer. The mean height of the crops at 10 weeks post application was 11.13±0.40 cm for treatment combination 2, 16.0±60 cm for treatment combination 3, 15.0±64 cm for treatment combination 4, and 18.50 ± 1.91 cm for treatment combination 5 (Figure 2). Ten weeks after planting, treatment combination 5 showed maximum plant height as compared to other treatment combinations. The results prove that addition of fertilizer gives dual effect- one, by stimulating the microbial growth, and second, by providing nutrients essential for plant growth.

Microbial population of soil

Table 5 shows reduced levels of microbial population two and ten weeks after planting as a result of crude oil application alone. The microbial population dropped from 0.46±0.02 × 10^4 cfu/g by the end of two weeks to 0.24±0.01 × 10^4 cfu/g at 10 weeks after inoculation. Crude oil application, thus, not only increased the toxic level leading to decrease in microbial population but also, impaired the availability of nutrition to microbes. However, the treatment combinations 3 and 4 raised the microbial population from 1.63±0.06 × 10^4 cfu/g at the end of two weeks to 4.5 × 10^4 cfu/g at the end of ten weeks (treatment combination 3) and further 3.33±0.12 × 10^4 cfu/g at 10 weeks after inoculation from 1.50 ± 0.05 × 10^4 cfu/g after two weeks (treatment combination 4). The treatment combination 5 showed a high number of microbial population from 2.20 × 10^4 at two weeks to 9.35±0.28 × 10^4 cfu/g at ten weeks after inoculation. The increased microbial population has stimulatory effects of the fertilizer on the proliferation of microorganisms (Song and Barth, 1990; Edosomwan et al., 1995). Highest

Table 5. Microbial population of the soil at 2, 6 and 10 weeks post inoculation with microbes under the six treatment combinations.

Treatment	Microbial growth (× 10^4 cfu/g)	
2 weeks	**6 weeks**	**10 weeks**
0.61±0.02	0.63±0.02	0.65±0.02
0.46±0.02	0.34 ±0.02	0.24±0.01
1.63±0.06	3.33±0.08	4.55±0.15
1.50±0.05	2.04±0.07	3.33±0.12
2.20±0.10	6.55±0.31	9.35±0.28
0.70±0.03	2.60±0.07	4.20±0.14

Treatment 1 = control without crude oil, Treatment 2 = soil + crude oil, Treatment 3 = soil + crude oil + microbes, Treatment 4 = soil + crude oil + fertilizer, Treatment 5 = soil + crude oil + microbes + fertilizer, Treatment 6 = soil + fertilizer.

population was a result of combination of microbes and fertilizer (treatment combination 4) at the end of ten weeks. This indicates that microbes and fertilizers added in combination, than singly, remediate contaminated soils effectively. The treatment combination 5 shows significant positive relationship with plant height along with effective crop emergence.

DISCUSSION

The study performed, has shown that the treatment combination 5, that is, soil, crude oil, microbes and fertilizer gave the highest plant height and percent crop emergence as compared to any other treatment combinations with crude oil. This suggests that addition of fertilizer gives dual effect- one, by stimulating the microbial growth, and second, by providing nutrients essential for plant growth. This implies that the expected negative effect of the pollution might have been remediated by the presence of the bacteria. More so, it appeared that fertilizer application does not only provide required nutrients for plant growth but it also has a stimulating effect on microbial growth and activity for bioremediation. This study is, to the best of our knowledge, the first one in the literature in which such type of evidences are collected from hilly areas of Uttarakhand.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Alexander M (1978). Introduction to Soil Microbiology., Fourth (ed). John Wiley and Sons New York. pp. 19-50.

Amadi A, Bari YI (1992). Use of poultry manure for amendment of oil polluted soil in relation to growth of maize. Environ. Inter. 18:521-527.

Amadi A, Dickson AA, Matte CO (1993). Remediation of polluted soil I. Effect of organic and inorganic nutrient supplements on the performance of Maize (*zea mays*). Water, Air Soil Pollut. 66:59-76.

Amakiri JO, Onofeghara FA (1984). Effects of crude oil pollution on the growth of *zea mays*. Oil Pet. Pollut. 1(3)199-206.

Anoliefo GO, Okoloko GE (2000). Comparative toxicity of Forcados Blend Crude Oil and its water soluble fraction on seedlings of *Cucumeropsis manni naudin*, Nigeria. J. Appl. Sci. 18:39-40.

Anoliefo GO, Vwioko DE, Mpamah P (2003). Regeneration of *Chromolaena odorata* (1) K. and R. in crude oil polluted soil: A Possible Phytoremediating Agent. Benin Sci. Dig. 1:78-82.

Bossert I, Bartha R (1984). The fate of petroleum in soil ecosystem. Pet. Microbiol. Mac-millan publisher Co. New York. pp. 436-473.

Dibble JT, Bartha R (1979). Effect of environmental parameters on biodegradation of oil spillage. Appl. Environ. Microbiol. 37:729-739.

Dorn PB, Salanitro JP (2000). Temporal ecological assessment of oil contaminated soils before and after bioremediation. Chemosphere 40:419-426.

Edosomwan NL (1995). Effect of phosphate fertilizer in retaining crude oil polluted soils. Niger. Ann. Nat. Sci. 2:169-177.

Gibson DT, Saylor GS (1992). Scientific foundations of bioremediation: Current status and future needs. AAM, New York.

Hanson KG, Nigam A (1997). Bioremediation of crude oil contamination with *Acinetobacter* sp. A3. Curr. Microbiol. 35(3)191-193.

Hoff R (1992). Bioremediation, A countermeasure for oil spills. Spill Technol. Newsletter 17:1-14.

Isirimah NO, Zoufa K, Loganathan R (1989). Effect of crude oil on maize performance and soil chemical properties in the humid forest zone of Nigeria. Discov. Innovat. 1(3)95-98.

Iyagba AG (2014). Effect of crude oil and biostimulant (bioremediation) on growth extract of Maize (*Zea Mays* (L.) and Cowpea (*Vignaunguiculata* (L.) Walp). Eur. Sci. J. 10:284-291.

Kyung-Hwa B (2004). Effect of crude oil, oil components, and bioremediation on plant growth. J. Environ. Sci. health. 39(9)2465-2472.

Lovley DR, Lloyd JR (2000). Microbes with mettle for bioremediation. Nat. Biotechnol. 18:600-601.

McCauley A, Jones C, Jacobsen J (2009). “Soil pH and organic matter” in Nutrient Management Module, Montana State University

Extension, Bozeman, Mont, USA. 8:1-12.
Nelson EW, Sommer LE (1982). Total carbon, organic carbon and organic Matter. Method of soil analysis. Agronomy Monographs 9, Madison. pp. 539-576.
Njoku KL, Oboh BO, Akinola MO, Ajasa AO (2012). Comparative effects of *Abelmoshus esculetus* (L) Moench (Okro) and *Corchorus olitorius* L (Jew Mallow) on soil contaminated with mixture of petroleum products. Res. J. Environ. Earth Sci. 4(4):413-418.
Nwogu TP, Azubuike CC, Ogugbue CJ (2015). "Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with Capra *aegagrus hircus* (Goat) Manure." Biotechnol. Res. Int. (2015):657349.
Odu CTI (1981). Degradation and Weathering of crude oil under tropical condition in petroleum industry and the Nigeria environment. Proceeding of the 1981 International seminar of the Nigeria National Petroleum Corporation.
Ogbonna DN, Iwegbue CMA, Sokari TG, Akoko IO (2007). Effect of bioremediation on the growth of Okro (*Abelmoshus esculetus*) in the Niger delta soils. Environ. 27(2)303-309.
Olsen SR, Sommer LE (1982). Phosphorous in A.L.Page (ed) Method of soil analysis. Second Agronomy Monographs 9, ASA and SSA, Madisen. pp. 403-403.
Olusola SA, Anslem EE (2010). Bioremediation of a crude oil polluted soil with *Pleurotus pulmonarius* and Glomus mosseae using *Amaranthus hybridus* as a test plant. J. Biol. Biodegrad. 1:113.
Sayles GD (1999). Land treatment of PAH-contaminated soil: performance measured by chemical and toxicity assays. Environ. Sci. Technol. 33:4310-4317.
Song HG, Bartha R (1990). Effect of jet spill on microbial community of soil. App. Environ. Microbiol. 58:646-651.
Sverdrup GD (2003). Toxicity of eight polycyclic aromatic compounds to red clover, ryegrass, and mustard. Chemosphere 53:993-1003.
Udo EJ, Fayemi AAA (1975). The effect of oil pollution in soil on germination, growth and nutrient uptake of corn. J. Environ. Qual. 4:537-540.
Uqab B, Mudasir S, Qayoom A, Nazir R (2016). Bioremediation: A Management Tool. J. Bio. Biodegrad. 7:331.
Zobell CE (1964). The occurrence, effect and fate of crude oil polluting the sea. Adv. Water Poll. Res. 3:85-118.