

Potential of *Cyperusrotundus* for Remediating Soils Polluted with Spent Engine Oil: Changes in Soil Chemical and Microbial Properties

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ABSTRACT: Recently, the use of plants to cleanup of crude oil contaminated soils has been a subject of increasing investigation. To identify native plants for the remediation of oil contaminated soil, the growth of *Cyperusrotundus* L. was observed under different concentrations (0, 50, 100, 150 and 200 g/kg) of the spent engine oil in pot experiment. Soil analysis showed that the oil had little effects on pH, exchangeable Na and K as well as texture but the organic C, N and total hydrocarbon (THC) increased relative to the control while concentrations of P, Ca and Mg decreased. The microbial population decreased with increasing concentration of the spent engine oil. Survival rates and dry matter yield of *C. rotundus* were considerably ($P < 0.05$) reduced by soil pollution. Overall, *C. rotundus* had satisfactory potential for growth if soil contamination does not exceed 10%. Further research should screen more native plants with remediating ability.

INTRODUCTION

Crude oil exploration in Nigeria has led to the pollution of land and water ways, especially in the oil producing belt. Due to its toxicity, widespread presence and complex nature, this type of pollution is a serious problem mainly because modernization and urbanization have led to increasing use of petroleum and petroleum-based products. Contamination of soil with oil is becoming an ever increasing problem due to several breakdowns of oil pipelines and wells, negligence when transporting, collecting or storing and careless disposal of old or used

petroleum products. The disposal of spent engine oil (SEO) also known as used engine oil into gutters, water drains, open vacant plots and farms is a common practice in Nigeria, especially by motor mechanics. This spent oil is usually obtained after servicing and subsequent draining from automobiles and generator engines (Anoliefo and Vwioke, 2001) and much of it is poured into the soil.

There are relatively large amounts of hydrocarbons in the used oil, including the highly toxic polycyclic aromatic hydrocarbons (Wang, *et al.*, 2000). Also, most heavy metals

such as V, Pb, Al, Ni and Fe which are below detection in unused lubricating oil have been reported at high values in used oil (Whisman, *et al.*, 1974). These heavy metals may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations and/or bound to organic matter (Young, *et al.*, 1992). Ekundayo, *et al.* (1989) and Eneje, *et al.* (2011), reported marked changes in the physico-chemical properties of soils polluted with petroleum hydrocarbons. Oil pollution of soils leads to build up of essential (organic C, P, Ca, Mg) and non-essential (Mn, Pb, Zn, Fe, Co, Cu) elements (Vwioke *et al.*, 2006) in the soil and the eventual translocation of metals to plant tissues. Although some heavy metals at low concentration are essential micronutrient for plants, at high concentrations, they may cause metabolic disorders and growth inhibition for most plant species (Fernandes and Henriques, 1991). However plants respond differently to pollutants. Anoliefo and Vwioke (1995) reported that the contamination of soil with spent engine oil caused growth retardation in plants. Soils polluted with petroleum-based products lose their biological activity and may not be able to recover it over ten years (Wyszkowska *et al.*,

2002; Sparrow and Sparrow, 1988; Racine, 1993).

Physical, chemical and thermal processes are the common techniques used in the cleaning up of oil contaminated sites (Frick *et al.*, 1999). These techniques however, have some adverse effects on the environment and are also expensive (Frick *et al.*, 1999; Laundsteat, 2003). Recently, biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum (Stephen *et al.*, 2013, Otura *et al.*, 2013). Phytoremediation relies on plants and or associated microorganisms to remove, contain or render harmful materials less hazardous (Merkl, 2005). It has been shown to be effective for different kinds of pollutants like heavy metals (Aliyue *et al.*, 2014), radionuclides and a broad range of organic pollutants (Schroder *et al.*, 2002; Schnoor, 2002). Plants such as *Jatropha curcas* (Idowu and fayinminnu; Abioye *et al.*, 2012; Chang *et al.*, 2014), *Lemna minor* L, *Scirpus grossus* (Al-Baldawiet *et al.*, 2015), ornamental plant species (Xiao *et al.*, 2015) etc. have been reported to possess phytoremediation abilities. According to Pivetz (2001) and Hattabet *et al.*, (2013), plants for phytoremediation should be appropriate for the local climate and soil

conditions of the contaminated sites. Such plants should also have the ability to tolerate conditions of stress (Siciliano and Germida, 1998). *Cyperusrotundus*, a tropical weed has been observed to possess such qualities but it has not been scientifically studied. Our objective was to determine the effectiveness of *Cyperusrotundus*, an indigenous weedy sedge to phytoremediate soils contaminated with spent engine oil based on changes in soil chemical and microbial properties.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the greenhouse of the Faculty of Agriculture, University of Calabar ($5^{\circ}3^1 - 04^{\circ}21^1$ N; $07.15^{\circ} - 28^1$ E), Nigeria. The annual temperature in the area ranges from 21 to 30°C while annual humidity is 70 to 85% (FDALR, 1987). Annual rainfall is very high (2000 -3500 mm) and is spread between March and October.

Soil and oil pollution treatment

The bulk soil was collected from the University of Calabar Teaching and Research farm, air-dried, crushed and sieved through a 4 mm mesh. Two kg of the sieved soil was weighed into

plastic pots of 3 L capacity. The treatments (spent engine oil) were applied at 0, 50, 100, 150 and 200 g/kg by adding no, 100, 200, 300 and 400mL of the spent engine oil to the soils in the pots. These high ranges of pollution were deliberately chosen to mimic common occurrence of large-scale oil spillage/pollution ($>10\%$) in Nigeria, which has rendered farmlands totally unproductive and devoid of vegetation. The oil was mixed thoroughly with the soil inside the plastic pots with the aid of a hand trowel. Treatments were replicated 5 times and arranged into a completely randomized design. Pre-treatment soil samples (screened via 0.5 or 2 mm sieve) were taken for microbial and physicochemical analysis.

The Experimental Plant

The test plant was *Cyperusrotundus*, a common weed in over 90 countries and the world's most invasive weed based on its distribution and effect on crops (Sharma and Gupta, 2007). It is native to Africa, Southern and Central Europe and Southern Asia. The plant belongs to the family Cyperaceae and is characterized by a complex underground network of tubers, basal bulbs, roots and rhizomes that ensure its ability to survive and reproduce during adverse conditions

(Cristina *et al.*, 2005). Further biological features such as its adaptation to high temperatures, solar radiation and humidity have turned the weed into a serious problem in subtropical and even arid regions. *Cyperus rotundus* seedlings of uniform height (about 15cm) were transplanted into each pot at 5 seedlings per pot.

Laboratory analysis

Soil samples were analysed for pH in a 1: 2.5 soil – water solution, using a pH meter with glass electrode. Organic carbon was determined by the Walkley-Black wet oxidation method as described in Nelson and Sommers (1982). Total nitrogen was determined using macro-kjedahl method as outlined in Bremner and Mulvarey (1982). Available phosphorus was determined by the Bray P-1 method (Bray and Kurtz, 1945).

Exchangeable bases were leached with ammonium acetate (NH_4OAc) at pH 7. The Ca^{2+} , Mg^{2+} , K^+ and Na^+ in the leached solution were determined by atomic absorption spectrophotometry. Exchangeable acidity was determined by first extracting soil with potassium chloride and titrating with 0.01 Molar NaOH using Phenolphthalein indicator (Udo, 2009). The effective cation exchange capacity (ECEC) was calculated as the sum of

exchangeable acidity and exchangeable bases.

Base saturation was obtained as the ratio (%) of total exchangeable bases to ECEC.

Microbial and TPH analyses

Soil samples were analysed for culturable microbial population (Zuberer, D. A. 1994). Probable organisms were identified using gram reaction, biochemical characterization and sugar fermentation and other standard procedures (Schmidt and Gier, 1989). For TPH analysis, 10 g of the polluted soil were extracted with dichloromethane. Impurities in the extract were removed with sodium sulphate and silica and analysed by GC/FID (Analysis of Petroleum Hydrocarbons in Environmental Media, Total Petroleum Hydrocarbon Working Group Series, March 1998).

Survival Rate and dry matter yield of the plants

We counted the number of plants per pot that survived after the experiment. Dry matter yield was obtained by oven-drying the above ground plant biomass at 60°C for 48 hours followed by weighing on an electronic scale.

Analysis of Data

The data were analysed using analysis of variance (ANOVA) and treatment means were compared using the least significant difference (LSD) at the 5% level of probability.

Results and discussion

Soil physicochemical characteristics

The soil (loamy sand) typically has excellent drainage but has low base saturation on account of the high rainfall (about 3000 mm per annum) in the area. The pH across treatments ranged between 5.2 and 6.4 and there was no significant difference between the control and polluted soils as reported previously (Okonokhuaet al., 2007). Soil organic carbon and total nitrogen increased significantly with increasing concentration of spent oil (Tables 1), essentially because these are principal natural constituents of engine oil (Okonokhuaet al., 2007). As a result, there was a marked difference ($P \geq 0.05$) between the control and contaminated soils both before planting and after harvest. Organic carbon ranged between 1.16% and 2.80% and total nitrogen between 0.1 in control soil and 0.25% in soil polluted with 400 mL of spent engine oil. However, available P and Mg decreased with increasing pollution. The soil P ranged and between 35.25 mg kg^{-1} and 46.0 mg kg^{-1} , being higher than the critical level of 20 mg kg^{-1} for crop production in the study area.

Except at the 400mL pollution level where ECEC and base saturation decreased

considerably, Ca and K levels were little affected by pollution. In a previous study, the low Ca^{2+} and Mg^{2+} contents of an oil contaminated soil caused a discoloration of plants leading to low yield (Nwilo and Badojo, 2005). Table 1 also shows a higher level of total hydrocarbon (THC) contamination in the treated soil. Such high level affects both above ground and subterranean flora and fauna (Osujiet al., 2004), which are essential adjuncts in the biogeochemical cycle affecting the availability of plant nutrients.

Soil microbial characteristics

The physical, chemical and biological characteristics of a particular soil as well as growing plants influence the number and activities of its various microbial components (Germida, 1993). The characterization and identification of bacterial, actinomycetes and fungal isolates respectively in this experiment are shown in Tables 2 and 3.

The microbial communities identified during the experiment were bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Arthrobacterglobiformis*, and *Achronobacterspp.*), Actinomycetes (*Actinomycete spp.*, *Streptomyces albus*,

Nocardiaspp.) and Fungi (*Rhizopusspp.*,

*Penicillium*spp., *Mucor* spp. and *Aspergillus*

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Table 1: Changes in soil physicochemical properties after harvest.

Treatment (mL)	pH	Organic C (%)	Total N (%)	Avail. P (mg/kg)	Exch. bases (cmol/kg)				Exch. Acidity	ECEC	Base Sat (%)	Texture (%)			TPH (mg/g)
					← Ca	Mg	K	Na →				Clay	Silt	Sand	
0	5.8	1.16	0.10	46.50	3.2	1.2	0.09	0.06	1.20	5.75	79.30	7.50	12.13	80.37	0.0
100	5.6	2.45	0.21	40.25	3.2	1.2	0.09	0.07	1.12	5.68	80.25	7.63	11.80	80.57	184.4
200	6.2	2.51	0.22	40.25	3.8	0.6	0.11	0.08	1.12	5.71	80.39	6.23	13.33	81.43	187.1
300	6.0	2.63	0.22	38.00	3.4	0.8	0.10	0.07	0.96	5.33	81.99	7.27	11.33	81.40	190.5
400	6.3	2.77	0.25	35.25	2.2	0.8	0.09	0.06	1.44	5.49	68.63	7.00	12.00	81.00	194.2
LSD (0.05)	-	0.05	0.04	2.15	0.12	0.13	-	-	0.07	0.16	9.00	-	-	-	7.0

Table 2: Characteristics and identification of bacterial and *actinomycetes* isolates.

Shape/ Arrangements	Gram stain reaction	Biochemical tests			Sugar fermentation				Probable organism
		Catalase	Oxidase	Motility	Glucose	Lactose	Manitol	Sucrose	
Rods in chain	+ve	-ve	-ve	+ve	A	A	A	A	<i>Acinobacter</i> spp
Rods in chain	+ve	+ve	-ve	+ve	-ve	A	A	A	<i>Bacillus subtilis</i>
Rods in chain	-ve	+ve	+ve	-ve	A	A	A	-ve	<i>Pseudomonas aeruginosa</i>
Cocci in chain	+ve	-ve	-ve	+ve	A	A	A	A	<i>Actinomycete</i> spp.
Rods in chain	+ve	+ve	+ve	+ve	-ve	A	A	-ve	<i>Achromobacter</i> spp.
Cocci in clusters	+ve	+ve	+ve	-ve	A	A	A	A	<i>Micrococcus</i> spp.
Rods and cocci	+ve	+ve	+ve	-ve	-ve	A	+ve	-ve	<i>Nocardia</i> spp
Rods in chain	+ve	+ve	.	-ve	+ve	+ve	+ve	+ve	<i>Streptomyces</i> spp

Table 3: Morphological characteristics and identification of fungi isolates

Colonial morphology	Nature of hyphae	Colour of hyphae	Arrangement	Spore	Probable organism
White with sulphur yellow colour	Non-septate	Brown	Single	Tiny spores, crowded	<i>Rhizopus</i> spp.
White colour with wavy appearance	Non-septate	Green	Single	Tiny spores, scattered	<i>Rhizopus</i> spp.
Round white colour	Non-septate	Brown	Intertwine	Tiny spores, scattered	<i>Mucor</i> spp.
Round green, with sulphur yellow colour	Non-septate	Brown	Intertwine	Tiny spores, scattered	<i>Aspergillus</i> spp.
Round green colour with wavy appearance	Non-septate	Brown	Single	Tiny spores, crowded	<i>Penicillium</i> spp.

Table 4: Mean microbial population

Treatments level	Bacteria & Actinomycetes Mean plate count (cfu/g)	Fungi Mean plate count (cfu/g)
0	152×10^{-6}	15×10^{-3}
50	137×10^{-6}	11×10^{-3}
100	124×10^{-6}	12×10^{-3}
150	106×10^{-6}	7×10^{-3}
200	87×10^{-6}	3×10^{-3}

microbial population could also result from influence by various factors such as pH, fertility level, moisture content, soil air, temperature, organic matter, ion concentration and cultural practices (Rangaswami and Bagyaraj, 1993).

Pseudomonas aeruginosa, *Bacillus subtilis*, *Aspergillus* spp. and *Penicilium* spp were observed to persist even at high concentrations (400ml) of spent engine oil. This is consistent with results of other researchers as bacteria, actinomycetes and fungi are known to be the principal agents of hydrocarbon biodegradation (Saadoun, 2008). The adaptive response of these organisms to the high level of spent engine oil in this experiment suggests that they have the physiological capabilities for survival in such extremely polluted soil. This adaptation is an indication that these persistent organisms may use the available substrate (spent engine oil) as an energy source and they may thus play vital roles to play in the degradation of contaminants. Leahy and Cowell (1990) reported that the rate of (petroleum) hydrocarbon degradation in nature is determined by the population of indigenous hydrocarbon degrading microorganisms, the physiological capabilities of these population plus other factors that may influence the growth of these degraders.

spp.). The microbial population decreased with increases in concentration of the spent engine oil (Table 4), possibly because freshly applied oil and/or high levels of contaminants often kill or inhibit a large sector of microbial biota (Saadoun, 2008; Dean-Ross 1984; Bosserd and Bartha, 1984). The decrease in the Plant growth and survival Exposure to oil contaminated soils affected the survival and biomass yield of the plant. The *Cyperusrotundus* showed characteristics of stunted growth, yellowing, poor stem development, wilting and death at high oil contamination levels (Table 5). Mortality was also significantly ($P \geq 0.05$) greater in soils with higher concentrations of spent oil. The average dry weight was much higher ($P \geq 0.05$) in the control than in the oil treated soils. The observations for plant growth and biomass yield were consistent with those of Udo and Fayemi (1975) and Daniel-Kalio and Tih (2003) even though they experimented on different plant species.

Table 5: Survival and biomass yield of *Cyperusrotundus* at different

concentrations of spent engine oil in the soil.

Treatment	Survival rate	Dry matter
	(%)	yield (g/pot)
0	100	8.84
100	72	7.83
200	52	6.27
300	28	4.39
400	16	2.04
LSD	23	0.46

Conclusion

This study has confirmed the negative impact of spent oil pollution on soils, which have accounted for the rapid destruction of vegetation and farmlands. The fertility status of the soils is reduced as the oil makes most of the essential nutrient unavailable for plant and crop utilization, possibly by immobilization, precipitation or complexation. The test crop (*C. rotundus*) showed characteristics of stunted growth, yellowing of plants, poor stem development, wilting and death at high oil contamination level but it may be possible to use it for remediating moderately or slightly (5-<10%) polluted soils in conjunction with organic amendment. Further research should compare different native plant species and at lower, more commonly encountered levels of pollution since

C. rotundus seems a poor phytoremediator at high levels of pollution.

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