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Phytoassessment of a Waste Engine Oil-polluted Soil Exposed to Two Different Intervals of Monitored Natural Attenuation Using African Yam Bean (*Sphenostylis stenocarpa*)

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Abstract: The present study comparatively investigated the phytotoxic effects of waste engine oil (WEO)-polluted soil exposed to monitored natural attenuation up to 5 and 14 months respectively. Soil was previously polluted with WEO at 0, 1, 2.5, 5 and 10% w/w oil in soil. Although, there was significant reduction in heavy metal concentration of soil as well as total hydrocarbon contents, performance of *Sphenostylis stenocarpa* was greatly retarded when sown at 5 months after pollution (MAP), with death of all seedlings except in the control. However, growth and yield performances were significantly ($p>0.05$) enhanced at 14 MAP. Computation of hazard quotient showed that ecological risk factor initially posed by the presence of heavy metals in the soil at 5 MAP was significantly ($p>0.05$) reduced to safe levels at 14 MAP.

Key words: Heavy metals, phytoassessment, polluted soil, *Sphenostylis stenocarpa,* waste engine oil

INTRODUCTION

Oil pollution is predominant feature in Nigeria, being a major oil-producing country. Pollution of this nature is either directly from crude oil or from Waste Engine Oil (WEO). This is even becoming very widespread and more devastating than crude oil pollution because it is a major source of pollution within communities, where it constitutes a major risk to both plants and animals, as well as soil organisms. The fact that motor mechanic workshops, as well as workshops of other artisans which use and dispose of WEO, occur in urban and rural areas, in bushes and open plots, means that gardens and farms in such areas are constantly under threat of pollution by WEO through run-offs which eventually deliver water soluble fractions of the contaminant.

Baker (1970), De Jong (1980) and Udo and Fayemi (1975) reported that oil in soil created unsatisfactory conditions for plant growth, thereby resulting in damage to cell membranes and leakage of cell contents as well as retarded growth, leaf chlorosis and plant dehydration in oil-polluted soil. Some plants have however been shown to be resistant to WEO (Anoliefo and Vwioko, 2001). Lower concentrations of WEO in soil have also been shown to stimulate plant growth (Anoliefo and Edegbai, 2000). This therefore presents the possibility for remediation of these contaminants by biological means. However, monitored natural attenuation generally describes the inherent capability of soils to reduce the concentration, mobility, toxicity and bioavailability of contaminants without any conscious human intervention. Over the years, this process has received widespread attention, particularly because contaminats are effectively remediated without human intervention. A number of physical and biological processes act in synergism to bring about successful remediation of contaminants. The present study therefore investigates the possibility for remediation of a waste engine oil polluted soil after exposure to two different intervals of monitored natural attenuation (5 and 14 months). The study also eventually assesses the toxicity of the polluted soil to African yam bean at the different times. African yam bean was selected for the phytotoxicity study considering the fact that, being one of the lesser-known legumes, it is gradually been explored for their possibilities of been exploited as a new plant resource to meet the growing needs of the human society.

MATERIALS AND METHODS

Top soil, 0-10 cm, (pH 5.58; total organic carbon 0.41%; total nitrogen 0.10%; electrical conductivity 300 μs/cm), was collected randomly from an area measuring 50×50 m on a farmland situated in the University of Benin Campus. Thereafter, 10 kg sun-dried soil was each placed into 50 large perforated 25 L paint buckets with 8 perforations made with 2 mm diameter nails at the bottom of each bucket. WEO was obtained from an auto-mechanic workshop in Ikpoba Hill, Benin City that specializes in repairs of heavy duty trucks/vehicles. The WEO was stored in 50 L jerry cans for use. Oil was added to soil in the buckets and mixed thoroughly to obtain

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5 different concentrations on weight basis: 0, 1.0, 2.5, 5.0 and 10.0% w/w oil in soil. The experiment was divided into two sets. The first set received African yam bean *(Sphenostylis stenocarpa)*, 5 months after pollution, whereas the second received *S. stenocarpa* after 14 months. *S. stenocarpa* was used as bio-test, using some growth and yield parameters as basis for comparison. The buckets containing the plant stands were rearranged on the field at a spacing of 60×30 cm, amounting to 55,000 stands/Ha (Ikhajiagbe *et al.*, 2007).

Soil physicochemical analyses: In the laboratory, soils were dried at ambient temperature (22-25°C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2mm fraction was used for the determination of selected soil physicochemical properties and the heavy metal fractions. Determination of organic carbon followed the methods of Osuji and Nwoye (2007). Soil total nitrogen was determined by the Kjeldahl Digestion Method. Determination of soil available phosphorus was according to Bray and Kurtz (1945). Exchangeable cations (Na, K, Ca and Mg) were determined following the methods of Tekalign *et al.* (1991), whereas determination of exchangeable acidity was according to Marschner (1986). Heavy metal fractions (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni, V) were determined by AAS according to the methods of APHA (1985).

Some growth and yield parameters determined in Cowpea.

Germination experiments: Percentage emergence was calculated as the percentage of seeds that sprouted above soil level of the 5 seeds originally sown per bucket. Heights were also taken by aid of a transparent calibrated ruler at 2 weeks after sowing (WAS). Fresh weights of seedlings were taken at 2 WAS by carefully uprooting the seedling and gently washing off the attaching root soils, air-drying the water and then weighing on a sensitive balance. Dry weights were obtained after drying seedlings in an oven at 30°C for 3 days.

Measurement of death of cowpea seedlings: Total number of cowpea seedlings that turned yellow and eventually became necrotic. Survival percentage was also calculated for seedlings at 2 weeks after sowing (WAS), being the percentage fraction of seedlings that survived at 2 WAS relative to total number of seedlings that originally sprouted.

Other measurable growth and yield parameters: Other measurable growth parameters included plant height, plant biomass and estimated grain yield which was calculated as the total weight of single seeds harvested per hectare, thus:

$$\text{Yield}(\text{kg ha}^{-1}) = \frac{100 \text{ seed wt. (g)}}{100} \times \frac{\text{pods/plant}}{1} \times \frac{\text{seeds/pod}}{1} \times \frac{55{,}000}{1{,}000}$$

Statistics: Mean was calculated for 20 determinations and analysis of variance was done using the SPSS-15 statistical software and means were separated by using the Least Significant Difference.

RESULTS AND DISCUSSION

Soil natural attenuation may have effected significant changes in the soil physicochemistry (Table 1). There was no significant change in soil pH over time. The most important destruction mechanism during natural attenuation is microbial mediated degradation. Non-destructive attenuation mechanisms include sorption, dispersion, dilution and volatilisation. During the former process the contaminants are broken down ultimately into harmless components such as water, chlorine and carbon dioxide. In the latter processes the contaminants remain but the concentrations in the groundwater decrease. Inorganic contaminants like heavy metals, arsenic and cyanide can be attenuated by chemical and physical processes but cannot be degraded. pH ranges were 5.46-5.58 from 1-14 MAP. The minimal decreases (in pH) observed may be attributed to the degradation of the hydrocarbons in the WEO which may have resulted in the release of acidic intermediate and final products that probably lowered pH of the mixture (Alexander, 1999). Electrical conductivity (EC) was significantly lower in the oil-affected soils than in the control soils. EC at 1 MAP was 293-308 μs/cm in the polluted soil as against 280 μs/cm in the control. At 14 MAP, there was reduction in EC to 189-210 μs/cm in the polluted soil compared to 139μs/cm in the unpolluted soil. This confirms the previous work of Osuji and Nwoye (2007). Concentration of soil potassium decreased with increasing pollution level, with a corresponding decrease in P. Generally, however, there was reduction in concentration of K and P at 14 MAP compared to values at earlier periods, possibly occasioned by remediation processes. Soils contaminated with petroleum products have been shown to have large increases in nitrogen and phosphate content (Odu, 1972; Amund *et al.*, 1993). Lehtomaki and Niemela

Table 1: Physicochemical parameters of waste engine oil-polluted soil at 1, 5 and 14 months after pollution

Time	pH	EC (μS cm^{-1})	TOC (%)	TN (%)	Ca (meq/100 g)	Mg (meq/100 g)	P (%)	Zn (ppm)	Cr (ppm)	Cd (ppm)	Pb (ppm)	V (ppm)	THC (ppm)
1MAP													
SP_0	5.53	280	0.40	0.23	15.04	10.93	110.2	38.9	1.8	ND	0.03	1.84	565
$SP_{1.0}$	5.50	293	0.51	0.34	15.23	10.32	98.6	46.3	2.7	0.01	0.58	2.98	4361
$SP_{2.5}$	5.49	279	0.57	0.21	14.91	10.60	102.5	52.7	3.2	0.02	1.08	3.28	6763
$SP_{5.0}$	5.48	295	0.64	0.23	15.21	10.73	98.5	66.5	3.9	0.03	1.73	3.73	8480
$SP_{10.0}$	5.50	308	0.69	0.20	15.44	11.91	98.9	78.6	4.8	0.04	2.30	4.06	9892
5 MAP													
SP_0	5.50	210	0.95	0.12	14.33	9.83	74.1	22.8	1.5	ND	ND	1.86	362
$SP_{1.0}$	5.46	258	0.86	0.18	13.91	8.90	69.8	36.3	2.3	0.01	0.45	2.06	3028
$SP_{2.5}$	5.49	271	0.91	0.21	14.10	8.72	72.3	47.8	2.6	0.02	0.80	2.12	4106
$SP_{5.0}$	5.46	263	0.89	0.16	14.53	9.18	70.1	56.3	2.8	0.03	1.41	2.48	7010
$SP_{10.0}$	5.50	280	0.99	0.13	14.82	9.33	82.6	68.6	3.8	0.03	2.08	3.48	8521
14 MAP													
SP_0	5.58+(0.90)	139-(33.81)	1.24+(30.53)	0.08-(33.33)	8.60-(39.98)	3.61-(63.33)	2.07-(97.21)	10.6-(53.51)	0.32-(78.67)	ND	ND	0.058-(96.88)	28.5-(92.13)
$SP_{1.0}$	5.60+(1.82)	189-(26.67)	1.60+(86.05)	0.23-(27.78)	9.36-(32.71)	3.86-(56.63)	2.13-(96.94)	18.7-(48.48)	0.69-(70.00)	ND	0.20-(55.56)	0.100-(95.15)	113.74-(96.24)
$SP_{2.5}$	5.62+(2.37)	220-(18.82)	1.56+(71.43)	0.34+(61.90)	10.12-28.23)	4.69-(46.22)	1.38-(98.09)	20.8-(56.49)	0.78-(67.14)	0.018-(60.00)	0.46-(42.50)	0.097-(95.42)	286.35-
93.03$SP_{5.0}$	5.58+(1.82)	195-(25.86)	1.39+(56.18)	0.29+(81.25)	8.07-(44.46)	4.01-(56.32)	1.59-(97.73)	23.1-(58.97)	0.92-(71.58)	0.012-(60.00)	0.78-(44.64)	0.123-(95.04)	425.98-
93.92$SP_{10.0}$	5.55+(0.91)	210-(25.00)	1.49+(50.51)	0.25+(92.31)	8.12-(45.21)	3.77-(59.59)	1.24-(98.50)	30.8-(55.10)	1.08-(71.58)	0.019-(36.67)	0.83-(60.09)	0.148-(95.75)	608.35-(92.86)

"SP" stands for WEO-polluted soil, whereas the adjoining subscripts represent the various percentage values of oil in soil; MAP: Months after Pollution; TOC: Total org. carbon; EC: Electrical conductivity; TN: Total nitrogen; EA: Exchangeable acidity, Numbers with +ve and –ve signs represent percentage gains and losses respectively compared to values from those at 5 MAP, ND: Not determine

Table 2: Germination parameters of *Sphenostylis stenocarpa* after Planting at 5 MAP

	Treatments				
Parameters	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
No. of days taken for emergence	3.5^b	3.8^b	4.3^b	5.3^{ab}	7.6^a
Percentage emergence (%) @ 1 WAS	80.34^a	78.23^a	62.23^{ab}	54.87^b	32.17^c
Height of emergent in 2 WAS (cm)	18.6^a	15.3^b	11.2^c	10.3^{cd}	8.4^d
Fresh wt. of emergents at 2 WAS (g)	0.963^a	0.708^a	0.573^{ab}	0.342^b	0.225^b
Dry wt. of emergents at 2 WAS (g)	0.443^a	0.401^a	0.307^{ab}	0.233^{ab}	0.196^b
Percentage survival of emergents at 2WAS	89.74^a	70.21^a	56.14^b	22.32^c	0^d
1st Day of noticed yellowing (DAS)	31.2^a	18.7^b	13.8^b	9.5^{bc}	8.2^c
Day of noticed necrosis in plant (DAS)	-	24.8^b	15.2^c	13.1^c	11.2^c
Day recorded total death of all seedlings (DAS)	-	28.2^b	18.7^c	16.3^{cd}	13.2^d

Values are means of 20 determinations. Means on the same rows with similar alphabets do not differ significantly ($p>0.05$) from each other. DAP: Days after planting; WAP: Weeks after planting. "SP" stands for the WEO-polluted soil, whereas the adjoining subscripts represent the various percentage values of oil in soil

(1975) reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contaminated soil. This confirms the discovery in this research.

Heavy metal concentration at 14 MAP (Table 1) decreased from original values at earlier periods irrespective of the level of pollution. At 14 MAP, soil concentration of Zn ranged from 10.6-30.8 ppm and from 0.07-0.13 ppm Ni. These value ranges were significant reductions from their original concentration at 1 MAP. Total hydrocarbon content at 1MAP was 431-9892 ppm in polluted soil as against 565 ppm in the control. This significantly decreased at 14 MAP to 113.74-608.35 ppm in polluted soil as against 28.5 ppm in the control. This represents over a hundred percent remediation in hydrocarbon content of soil.

The effects of oil pollution on the growth, development and performance of African yam bean may be very devastating. In the present study, percentage germination reduced according to increasing pollution levels at 5 MAP. At 14 MAP, however, when considerable level of remediation had taken place, percentage germination was improved. Table 2 presents the germination parameters of African yam bean planted after leaving polluted soil to lie fallow for 5 months. It took longer days for seedlings emergence with increased level of soil pollution, given the value at 7.6 days in the 10% w/w oil in soil treatment (SP_{10}) compared to 3.5 days in SP_0. At one week after sowing (1 WAS), percentage emergence of African yam bean seedling was 80.34% in SP_0 (control), 78.23% in SP_1 and 32.17% in $SP_{10,}$ inferring inhibited germination rate with increased oil pollution. At two weeks after sowing (2 WAS), African yam bean seedlings were 18.6 cm long in SP_0 and 8.4 cm long in the 10% w/w oil in soil treatment (SP_{10}). Chlorosis was first noticed in the control (SP_0) in 31 days after sowing, but the plant eventually recovered and greened up. However, yellowing was first noticed in the 1% w/w oil in soil (SP_1) treatment in 18 days; 14 days in $SP_{2.5}$, 10 days in SP_5 and 8 days in SP_{10} respectively. These plants also began to experience necrosis from the 25th day in the SP_1, 15th day in the $SP_{2.5}$ and 13th day in SP_5 and 11th day in SP_{10}. Within 28 days, all the plants in the pollute soil had died. Many authors (Udo and Fayemi, 1975; Amakiri and Onofeghara, 1983; Terge, 1984; Vwioko and Fashemi, 2005; Kayode *et al.*, 2009) have studied effects of oil pollution on seed germination of crop plants. Oil contaminated soil generally causes delayed seed emergence and that of WEO-contaminated soil is not different (Anoliefo and Vwioko, 1995). Terge, 1984) reported that germination of seeds in oil polluted soil varied with different plant species. Germination and seedling emergence of *Solanum melongena* and *S. incarnum* were inhibited by WEO. Germination of *Ricinus communis* in WEO-pollutedsoil was inhibited (Vwioko and Fashemi, 2005). Oil pollution retarded the growth of *Vigna* seedlings at 5 MAP.

This growth reduction was however lessened when *Sphenostylis* was sown at 14 MAP, after considerable amounts of soil pollutants would have been remediated by natural attenuation. The growth inhibition in the *S. stenocarpa* seedlings increased with the increase in the concentration of the used oil pollutants (Kayode *et al.*, 2009). However, germination was possible at 14 MAP (Table 3), as all seedlings advanced beyond the seedling stage, all the plants in $SP_{10.0}$ treatment soil eventually died just before flowering time due to persistent chlorosis. Shoot length at 8 WAS decreased from 124.36 - 78.68 cm. There were significant differences in the heights of the seedlings in the used oil polluted soils and those of the non-polluted soils. Grain yield in the control experiment was 1362.87 kg ha^{-1}. There was however significant reduction in yield of plants sown in the remediated soil, rainging from 804.34 kg ha^{-1} in SP1 to 391.65kg ha^{-1} in

Table 3: The effects of Soil Amendment on some Growth and Yield Parameters of *Sphenostylis stenocarpa* after 14 months

	Treatments				
Parameters	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
No. of days taken for emergence	3.4^{a}	3.9^{a}	3.7^{a}	5.3^{a}	4.6^{a}
Percentage emergence (%) @ 1 WAS	82.34^{a}	78.23^{a}	70.23^{ab}	64.54^{b}	62.32^{b}
Height of emergent in 9DAS (cm)	17.8^{a}	16.3^{a}	14.5^{ab}	11.6^{b}	10.7^{b}
Dry wt. of emergents at 9DAS (g)	0.406^{a}	0.389^{a}	0.311^{a}	0.263^{b}	0.201^{b}
Percentage survival of emergents at 2WAS	88.74^{a}	84.21^{a}	78.14^{a}	54.13^{b}	58.15^{d}
Shoot height (cm) @ 8WAP	124.36^{a}	101.27^{b}	96.42^{b}	95.82^{b}	78.68^{c}
No. of primary branches@8WAP	16.11^{a}	14.23^{a}	13.21^{ab}	12.98^{a}	7.98^{b}
Stem width (mm) @8WAP	8.13^{a}	8.02^{a}	7.76^{a}	7.01^{a}	5.02^{b}
Leaflet area (cm^2) @8WAP	53.01^{a}	50.21^{b}	50.12^{b}	49.21^{b}	38.20^{c}
No. of primary root branches/plant@8WAP	5.89^{a}	5.23^{a}	4.97^{a}	4.01^{ab}	3.45^{b}
Root length (cm) @8WAP	54.64^{a}	48.12^{b}	49.21^{b}	47.21^{b}	40.41^{c}
Shoot dry Wt. (g) @8WAP	12.67^{a}	11.51^{a}	10.43^{ab}	8.42^{bc}	7.54^{c}
No. of root nodules/plant (> 3mm in diameters)	22.58^{a}	17.42^{b}	15.65^{b}	16.21^{b}	10.67^{c}
Av. Nodule dry wt. ($x10^{-2}$ g)	9.48^{a}	7.23^{b}	7.11^{b}	6.34^{bc}	5.41^{c}
Days to 50% flowering (DAP)	75.42^{a}	78.12^{a}	82.09^{b}	81.02^{b}	Nil^{c}
Days to 50% maturity (DAP)	84.75^{a}	89.95^{b}	93.24^{c}	93.12^{c}	Nil^{d}
No. of flowers/plant	106.79^{a}	91.01^{ab}	88.23^{b}	81.22^{c}	Nil^{d}
No. of pods/plant	21.69^{a}	18.92^{b}	13.11^{c}	11.12^{c}	Nil^{d}
No.of seeds/pods	13.28^{a}	10.21^{b}	9.03^{b}	8.21^{b}	Nil^{c}
100 seed wt (g)	9.01^{a}	8.09^{a}	7.78^{a}	8.01^{a}	Nil^{b}
Grain yield (kg ha^{-1})	1362.87^{a}	804.34^{b}	481.14^{c}	391.65^{c}	Nil^{d}

Values are means of 20 determinations. Means on the same rows with similar alphabets do not differ significantly (p>0.05) from each other. DAP: Days after planting; WAP: Weeks after planting. "SP" stands for the WEO-polluted soil, whereas the adjoining subscripts represent the various percentage values of oil in soil

SP5 treatments respectively. Plants in SP10 treatments, as earlier reported, died just before flowering and hence no yield. Nwoko *et al.* (2007) reported suppressed growth and reduced yield in *Phaseolus vulgaris* grown in a soil contaminated with spent-engine oil.

The presented study shows that growth and development of African yam bean was possible having been exposed to prolonged monitored natural attenuation. Natural attenuation affords the soil the opportunity for the allowance of its wealth of biological and physicochemical processes in remediating the soil. The present study has successfully demonstrated that bioremediation can occur by natural attenuation.

Under such conditions as sorption or oxidation-reduction reactions, natural attenuation may effectively reduce the dissolved concentrations and/or toxic forms of inorganic contaminants in the soil. Both metals and non-metals may be attenuated by sorption reactions such as precipitation, adsorption on the surfaces of soil minerals, absorption into the matrix of soil minerals, or partitioning into organic matter. Natural attenuation processes typically occurs at all areas in the soil, but to varying degrees of effectiveness depending on the types and concentrations of contaminants present and the physical, chemical and biological characteristics of the soil as shown in the present study.

CONCLUSION

The present study thus showed that natural attenuation can effectively reduce contaminant concentration in the soil without direct anthropogenic intervention. The longer the time provided for such processes, the more effective the contaminant remediation. The study also provided information on the possibility of sowing crop plants in oil-polluted soil only after considerable amount of time has been allowed for such soils to naturally attenuate when other remediative options were unavailable.

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