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Phytoassessment of a 5-Month Old Waste Engine Oil Polluted Soil after Augmentation with *Pleurotus tuberregium*

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Abstract: The present study is a bioassessment of the effects of of substrate microbial augmentation on the bioremediation of Waste Engine Oil (WEO) polluted soil. Four different concentrations of WEO in soil on weight basis were obtained by thoroughly mixing WEO in measured soil: 1.0, 2.5, 5.0, and 10.0% w/w. The unpolluted soil was used as the control (0% w/w) experiment. The set up was left for 5 months without physically disturbing the soil. After 5 months, the soils were first amended with sawdust and then inoculated with mycelia of *Pleurotus tuberregium.* Nine months after bioaugmentation (9 MAB) there was total (100%) remediation of some PAH compounds (benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, and indeno(1,2,3-c,d)pyrene) was recorded. Significant ($p = 0.05$) decreases in heavy metal concentration from 5-9 MAB resulted in significant reductions in Hazard Quotients (HQ), which implied less possibility for ecological risk for heavy metal constituents. Phytoassessment of the polluted soil was carried at 5MAP, and results showed that virtually all the cowpea seedlings died within 2 weeks. Only those seedlings in unpolluted soils survived. Nine months after readjustment of soil treatments, all cowpea plants survived up to fruiting, with grain yields in the most polluted soil being 15.25 g/plant compared to 26.01 g/plant in the control experiment. Although heavy metals were minimally accumulated in leaves and seeds of cowpea, bioaccumulation was not significant when Bioaccumulation Quotients (BQ) were computed, with BQ value ranges as 0.01-0.05 in seeds and 0.0 -0.80 in leaves. Hydrocarbons was detected in cowpea leaves but not in the seeds.

Key words: Bioaugmentation, phytoassessment, *Pleurotus tuberregium*,waste engine oil

INTRODUCTION

The poor economic situation in many developing countries has led people to seek alternative employment as artisans including mechanics. Activities of these mechanics include changing oil from engines, machines and generators. They (including the users) indiscriminately disposal of the waste engine oil (WEO) into gutters, water drains, open vacant plots and farmlands. The result is the pollution of soil that has led to toxicity of crop plants as well as reduced growth and productivity. Human and animal toxicity may also occur indirectly as WEO consists of heavy metals and hydrocarbons (Alexander, 1999). The processes, therefore, leading to the eventual removal of these pollutants from the environment involves physical, chemical and biological alternatives. Physical and chemical methods are not simple or favourable because they further introduce poisonous contaminants to the environment. Therefore, bioremediation of these polluted soils only becomes imperative. For efficient bioremediation, however, soil amendments are added to increase micro-organisms activities (Davis and Wilson, 2005). *P. tuberregium* has been used in the experimental bioremediation of soils contaminated with crude oil (Colombo *et al*., 1996; Isikhuemhen *et al*., 2003; Adedokun and Ataga, 2007), and have also been noted to have the ability to increase nutrient contents in soils polluted with 1-40% engine-oil concentration after six months of incubation (Adenipekun, 2008). It is therefore, the aim of the present study to find out the impact of substrate microbial augmentation on the bioremediation a 5 month old waste engine oil-polluted soil by using viable mycelia of *Pleurotus tuberregium.*

MATERIALS AND METHODS

Top soil (0-10 cm), of known physicochemical properties (Table 1), was collected randomly from a marked area measuring 50 × 50 m on a farmland situated in the University of Benin Campus, Benin City, Nigeria. Thereafter, 10 kg sun-dried soil was each placed into 50 large perforated 25-;L buckets with 8 perforations made with 2 mm diameter nails at the bottom of each bucket. Waste Engine Oil (WEO) was obtained from auto-mechanic workshops in Benin City, and then pooled together to obtain a composite sample. Oil was added to soil in the buckets and mixed thoroughly to obtain 4

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Table 1: Physical and chemical properties of soil and sawdust used before soil contamination

Parameters	Units	Soil	Sawdust
pH	-	5.58	5.59
EC	µs/cm	300	330
TOC	%	0.41	1.99
Total Nitrogen	%	0.10	0.15
EA	meq/100 g soil	0.20	0.30
Na	meq/100 g soil	10.90	11.10
K	meq/100 g soil	1.65	2.65
Ca	meq/100 g soil	15.60	22.30
Mg	meq/100 g soil	11.30	15.40
Cl	mg/L	1666.00	1347.00
P	mg/L	153.00	112.00
NH_4N	mg/L	25.40	6.25
NO_2	mg/L	15.01	25.99
NO_3	mg/L	30.75	92.23
SO_4	mg/L	14.63	14.95
Clay	%	4.4	0
Silt	%	7.8	0
Said	%	87.8	0
Fe	mg/L	1009	220
Mn	mg/L	17.00	41.70
Zn	mg/L	30.00	4.80
Cu	mg/L	3.90	1.60
Cr	mg/L	2.18	1.60
Cd	mg/L	N.D	N.D
Pb	mg/L	0.03	N.D
Ni	mg/L	3.60	0.70
V	mg/L	1.36	0.64
THC	mg/L	754.00	268.00

ND: Not determined (<0.0001 mg/L)

different concentrations on weight basis: 1.0, 2.5, 5.0, and 10.0% w/w oil in soil (Ikhajiagbe and Anoliefo, 2010). Unpolluted soil was used as the control (0% w/w) experiment.

The entire set up was left for 5 months, without mechanically disturbing the soil. Soil was carefully irrigated daily (at dusk) with 400ml of water. After 5 months, 3 kg of soil was removed from each bucket, and replaced with 3 kg air-dried sawdust of known physicochemical property (Table 1), obtained from *Brachistegia nigerica*. Sawdust of *Brachistegia nigerica* was preferred because of its ability to enhance growth and performance of *Pleurotus tuberregium* (Okhuoya *et al*., 1998).

Production of mycelia: A well grown mushroom with membrane covering the gills was selected from which a small amount (0.5 g) of mushroom from gill portion was taken using forceps and inoculated on PDA media slants under aseptic conditions The mycelium covered the entire surface of the Petri dish in about a week's time and culture was ready for further multiplication.

Very good quality sorghum grains, free from pest and moulds, were selected, and then boiled, submerged in clean water for 20-30 min. When the grains became soft, they were removed and spread evenly on a cotton cloth to drain out the water and cool the grains. The soft grains were later mixed with 3% chalk powder (30 g/kg of grain) for adjusting the pH and to keep the grains loose. 250 g of grain of this mixture, each, was then poured unto cleaned and dried glucose bottles of 500 mL capacity each, with the mouths of the bottles tightly plugged with non absorbent cotton wool. The bottles were sterilized in an autoclave at 121ºC for 60 min. After cooling, the bottles were transferred to the inoculation chamber, where few individual grains with mycelial growth, collected from the mother spawn, were then transferred into sterilized substrate bottle using sterilized forceps, under aseptic conditions and plugged with cotton wool. Care was taken so that Mother Spawns were never used beyond 3 generations. The inoculated bottles were transferred to spawn running at 25-30ºC. The bottles were inspected regularly, and in the process, contaminated bottles were immediately discarded. Within 15-20 days from inoculation, mycelial growth had covered the entire substrate and the spawn was kept in the refrigerator, and ready for use.

Segregation into treatment: The sawdust amended soils were then immediately inoculated with 600 g mycelia mass of *Pleurotus tuberregium.* Inoculated soil treatments at 5 Months After Pollution (MAP) were labeled SSM, while SP was used to designate the uninoculated polluted soil at 5 MAP. The subscripts represented the levels of concentration of oil in soil. The designations were therefore SP_0, SP_1, $SP_{2.5}$, SP_5 and SP_{10} as well as SSM_0, SSM_1, $SSM_{2.5}$, SSM_5 and SSM_{10}. Subscripts of either SSM or SP were percentage concentrations of oil in soil.

Soil physicochemical analyses: In the laboratory, soils were dried at ambient temperature (22-25ºC), crushed in a porcelain mortar and sieved through a 2mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm 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 (1945a, b). 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 Marscher (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 9 Days After Sowing (DAS). Fresh weights of seedlings were taken at 9 DAS 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 30ºC for 3 days.

Measurement of death of cowpea seedlings: Total number of cowpea seedlings that turned yellow and eventually became necrotic was recorded. 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, stem width, number of leaves, total leaflet area, main root length, number of primary root branches, number of root nodules per plant, plant/root dry weight, total number of flowers per plant, length and number of pods/plant as well as estimated grain yield.

Chemical and nutritional parameters of cowpea: Determination of total soluble carbohydrates contents as well as elemental nutrient analysis of cowpea were done according to the methods of IITA (1979).

Computation of hazard quotient for heavy metal toxicity to cowpea: Hazard Quotient (HQ) was computed to express the possibility of the contaminant being an ecological risk or a Contaminant of Potential Ecological Concern (COPEC) to cowpea. HQ is expressed by the following equation:

HQ = Measured concentration/Toxicity reference value or selected screening benchmark

when HQ > 1: Harmful effects are likely due to contaminant in question

when HQ = 1: Contaminant alone is not likely to cause ecological risk

when HQ < 1: Harmful effects are not likely

Computation of bioaccumulation quotient for heavy metal accumulation in cowpea: Bioaccumulation Quotient (BQ) expresses the possibility of the contaminant being significantly accumulated in plant parts, thereby posing health threats.

BQ = Concentration of accumulated pollutant in the accumulantConcentration of accumulated pollutant in Soil (Source)

when BQ>1 = Significant accumulation in of the pollutant is implied

when BQ<1 = Bioaccumulation is not of significant effect

Toxicological benchmarks for screening contaminants of potential concern for effects on cowpea were provided by Efroymson *et al.* (1997).

RESULTS AND DISCUSSION

Significant ($p<0.05$) decreases in heavy metal composition were recorded nine months after bioaugmenting (9 MAB) the 5 month old polluted soil (5 MAP) with *P. tuberregium* (Table 2). Fe content of soil was 266-698 mg/L at 9 MAB compared to 768-1389 mg/L before bioaugmentation. Zn content of soil was 10.6-30.8 mg/L at 9 MAB compared to 22.8-68.6 mg/L before bioaugmentation at 5 MAP. Also the Ni content of soil was 0.07-0.13 mg/L at 9 MAB compared to 2.5-4.2 mg/L at 5 MAP. Total hydrocarbon content of soil was 28.50-608.35 mg/L compared to 362-8521 mg/L at 5 MAP.

Table 2: Heavy metal composition and total hydrocarbon content of a 5-month old waste engine oil-polluted soil (5 MAP) after additional nine months of bioaugmentation with *Pleurotus tuberregium* (9 MAB)

←mg/L→											
Time	Code	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
5 MAP	SP_0	768	18.5	22.8	2.3	1.5	ND	ND	2.5	1.86	362
	$SP_{1.0}$	1039	30.2	36.3	3.2	2.3	0.01	0.45	2.6	2.06	3028
	$SP_{2.5}$	1063	35.6	47.8	3.8	2.6	0.02	0.8	3.2	2.12	4106
	$SP_{5.0}$	1096	36.9	56.3	3.7	2.8	0.03	1.41	4.2	2.48	7010
	$SP_{10.0}$	1389	38.7	68.6	4.2	3.8	0.03	2.08	4.1	3.48	8521
	$\sum^{(SS)}$	(1071)	(31.9)	(46.3)	(3.4)	(2.6)	(0.02)	(1.19)	(3.3)	(2.40)	(4605)
9 MAB (or 9 MAB)	SSM_0	266(-65.36)	30.8(+66.48)	10.6(-53.51)	0.71(-69.1)	30.32(-78.67)	N/D	ND	0.07(-97.20)	0.058(-96.88)	28.50(-92.13)
	$SSM_{1.0}$	429(-58.71)	36.7(-21.52)	18.7(-48.48)	0.73(-77.19)	0.69(-70.00)	N/D	0.20(-55.56)	0.10(-96.15)	0.100(-95.15)	113.74(-96.24)
	$SSM_{2.5}$	619(-44.77)	33.8(-5.06)	20.8(-56.49)	1.19(-68.68)	0.78(-67.14)	0.018(-60.00)	0.46(-42.50)	0.12(-96.25)	0.097(-95.42)	286.35(-93.03)
	$SSM_{5.0}$	785(-28.38)	25.2(-31.71)	23.1(-58.97)	1.31(-64.59)	0.92(-71.58)	0.012(-60.00)	0.78(-44.64)	0.10(-97.62)	0.123(-95.04)	425.98(-93.92)
	$SSM_{10.0}$	698(-49.75)	28.1(-27.39)	30.8(-55.10)	1.43(-65.59)	1.08(-71.58)	0.019(-36.67)	0.83(-60.09)	0.13(-96.83)	0.148(-95.75)	608.35(-92.86)

SP: waste engine oil-polluted soil; SSM: fungus-amended SP-soils; whereas the adjoining subscripts represent the various percentage values of oil in soil; MAP: Months after pollution; MAB: Months after augmentation of soil with Pleurotus tuberregium; TOC: Total org. carbon; EC: Electrical conductivity; TN: total nitrogen; EA: Exchangeable acidity; ND: Not determined (<0.0001 mg/L); Figures in parenthesis with +ve and –ve signs: percentage gains and losses respectively compared to values from those at 5 MAP

Table 3: Polyaromatic hydrocarbon content of a 5-month old waste engine oil-polluted soil (5 MAP) after additional nine months of bioaugmentation with *Pleurotus tuberregium* (9 MAB)

	5 MAP9					MAB (or 9 MAB)				
PAH (mg/L)	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}	SSM_0	SSM_1	$SSM_{2.5}$	SSM_5	SSM_{10}
Acenaphthene	0.6755	1.2332	1.8677	2.0987	2.7865	0.1756	0	0	0	0
Acenaphthylene	0.09987	1.9887	2.1111	2.7654	3.9033	0.2146	0.2995	0.3862	0	0.5356
Anthracene	0.0077	6.0222	8.9900	11.5676	15.6553	0.1835	0	0	0.3842	0
Benzo[a]Anthracene	0.0060	2.8010	3.9887	4.0005	5.7866	0	0	0	0	0
Benzo[a]Pyrene	2.9088	3.9821	7.3221	9.9900	11.1773	0	0.9716	0.1117	1.5243	0
Benzo[b]Fluoranthene	0	0.2096	0.8876	0.9654	2.8766	0	0	0	0	0
Benzo[g,h,i]Perylene	29.6008	107.8221	111.0626	116.5696	124.6672	0	0	0	0	0
Benzo[k]Fluoranthene	0.1008	39.4432	96.5432	198.7659	307.4366	0	0	0	0	0
Chrysene	0.0767	0.23343	0.3212	0.1655	0.1187	0	0	0	0	0
Dibenzo[a,h]Anthracene	0.1001	0.6554	0.8544	2.6554	2.9900	0	0	0	0	0
Fluoranthene	1.3574	11.5447	19.0776	30.6245	44.2233	0	0	0	0	1.1126
Fluorene	0	0.6554	0.9887	2.7555	2.9778	0	0	0	0	0
Indeno[1,2,3-c,d]Pyrene	0	0.2232	0.7665	1.7665	2.4445	0	0	0	0	0
Naphthalene	0.4044	0.4122	0.4533	0.4127	0.5007	0	0	0	0	0
Phenanthrene	1.7508	7.8776	11.0997	17.9887	27.3443	0.2812	0.6766	0.7032	0.8055	0.8638
Pyrene	3.1249	7.4553	18.4453	29/7098	46.3342	0	0	0	0	0.4632
Total	40.2138	192.559	284.78	403.092	601.223	0.8549	1.9478	1.2011	2.7145	2.9752

SP: waste engine oil-polluted soil; SSM: fungus-amended SP-soils; whereas the adjoining subscripts represent the various percentage values of oil in soil; MAP: Months after pollution; MAB: Months after augmentation of soil with Pleurotus tuberregium

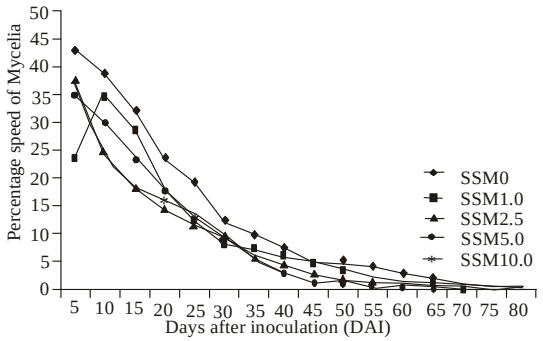

Fig. 1: Percentage Spread of Mycelia Mat per Soil surface Area of WEO-PoIIuted soil augmented with *Pleurotus tuberregium*

There were significant reductions in soil PAH (Table 3). Reductions were either total (100%) or significantly partial (50-98% reductions). PAH remediation in soil has been reportedly successful through microbial activity (Jordan and Payne, 1980; Yamazaki *et al*., 1988; Venkateswarlu *et al*., 1996). By comparison, of the entire individual PAH compounds identified in each soil treatment type, 66.22% of total individual PAH compounds were completely (100%) remediated. Chrysene, fluoranthene, and fluorene were totally remediated at 9 MAB irrespective of treatment soil, from initially highest soil concentration of 0.1006, 38.4333 and 0.6623 mg/L, respectively at 5 MAP. Light aromatic hydrocarbons are prone to evaporation and to microbial degradation in a dissolved state (Jordan and Payne, 1980). *Aspergillus niger* and *A. fumigatus* have both been reported to metabolize terpenes and PAHs; *A. niger* converts the terpene B-myrcene to dihydroxylated derivatives (Yamazaki *et al*., 1988). *A. fumigatus* also produces a cytochrome P_{450} that hydroxylates benzo[a]pyrene (Venkateswarlu *et al*., 1996).

Figure 1 shows the percentage spread of mycelia mat per soil surface area of WEO polluted soil in 80 days of inoculation. The spread of mycelia mat of *Pleurotus tuberregium* decreased with pollution level as well as with time. Total disappearance of mycelia was reached at 65 Days After Inoculation (DAI) in SSM_{10}, 70 days in $SSM_{2.5}$ and SSM_5, 80 days in SSM_1 and 83 days in SSM_0. The tolerance of the mycelia of *P. tuberregium* in the pollutants, for over 8 weeks, is therefore noted. Aggregation of mycelia into whitish 'puff' balls was generally recorded at 9-12 weeks after inoculation (WAI), which eventually deteriorated beyond 12 WAI. Presence of *Psathyrella atroubonata* was recorded exclusively to SSM treatments (15 in SSM_0, 12 in SSM_1 and 2.5, 8 in SSM_5 and 2 in SSM_{10}. The outstanding mycelia growth of *P. tuberregium* on all augmented (SSM's) treatments and at all concentrations used, may be due to higher production of extra cellular enzymes that enabled it utilize the hydrocarbons. The successful growth and reduction of total petroleum in crude oil-contaminated soil by *Pleurotus tuberregium* has been previously reported by Isikhuemhen *et al.* (2003). The findings of the present study agree with their research results of Isikhuemhen *et al.* (2003), although growth of *Pleurotus tuberregium* was eventually checked after 12 weeks. Mycelial mats are used for bioremediation because mycelia produce extra cellular enzymes and acids that break down recalcitrant molecules such as; lignin and cellulose and that lignin peroxidases dismantle the long chains of hydrogen and carbon making them effective at breaking apart hydro carbon the base structure common to oils, petroleum products, pesticides, PCB's and many other pollutants.

Table 4: Germination parameters of cowpea after planting in a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *Pleurotus tuberregium*

	No. Of Days taken for seedling emergence	Percentage emergence at 1 WAS (%)	Height of emergents at 9DAS (cm)	Fresh Wt. of emergents at 9DAS (g)	Dry Wt. of emergents at 9DAS (g)	Percentage survival of emergents at 2WAS	1st Day of noticed yellowing (DAS)	Day of noticed necrosis in plant (DAS)	Day recorded total death of all seedlings (DAS)
SP_0	3.8 (0.25)	82.14 (5.15)	14.6 (0.62)	0.763 (0.109)	0.249 (0.098)	82.14 (4.48)	19 (3)	0	0
$SP_{1.0}$	4.2 (0.24)	78.57 (4.68)	11.3 (0.68)	0.323 (0.098)	0.219 (0.058)	71.43 (5.02)	11 (2)	12 (3)	18 (2)
$SP_{1.6}$	4.8 (0.35)	64.29 (4.02)	9.7 (0.78)	0.428 (0.102)	0.200 (0.036)	57.14 (3.58)	10 (2)	13 (3)	18 (1)
$SP_{5.0}$	5.2 (0.58)	57.14 (3.56)	10.2 (1.03)	0.315 (0.099)	0.156 (0.029)	28.57 (4.92)	8 (3)	11 (2)	16 (2)
$SP_{10.0}$	7.6 (0.95)	28.57 (5.02)	7.6 (1.05)	0.218 (0.073)	0.117 (0.065)	0	9 (2)	11 (2)	13 (2)

Figures in parenthesis are standard deviations of the adjoining means for 10 determinations; DAS:Days after sowing; WAS:Weeks after sowing

Table 5: Some growth parameters of cowpea after planting in a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *Pleurotus tuberregium*

	Percentage emergence At 2 WAS (%)	Shoot height (cm)	Leaflet area (cm^2)	Root length (cm)	10 Nodule wt (g)	Plt dry wt. (g)
SSM_0	84.29 (8.54)	106.97 (11.21)	65.18 (5.22)	52.71 (6.2)	0.96 (0.23)	12.28 (2.24)
$SSM_{1.0}$	72.86 (9.57)	100.35 (10.55)	70.08 (6.87)	42.15 (6.00)	0.83 (0.22)	11.06 (2.54)
$SSM_{2.5}$	71.43 (8.65)	86.72 (9.98)	64.13(8.58)	48.15 (5.23)	0.93 (0.26)	11.73 (1.09)
$SSM_{5.0}$	58.57 (4.68)	78.63 (9.25)	63.85 (7.65)	42.98 (5.29)	0.78 (0.33)	12.02 (2.07)
$SSM_{10.0}$	47.14 (6.2)	85.42 (7.54)	58.67 (5.98)	38.43 (4.87)	0.86 (0.21)	11.18 (2.10)

Figures in parenthesis are standard deviations of the adjoining means for 10 determinations

Table 6: Yield parameters of cowpea sown in a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *Pleurotus tuberregium*

	Day of prod. of 1st Pod (DAS)	Day of 1st flowering (DAS)	No. of flowers /plt At 15WAS	Harvest day (DAS)	Pod/Plt	Pod length (cm)	Seed/Pod	100 seed wt. (g)	Yield/Plt (g/Plt)
SSM_0	67 (4)	59 (3)	48.85 (3.06)	90.18 (3.99)	15.26 (2.52)	13.96 (2.06)	11.93 (1.47)	14.29 (1.02)	26.01 (2.00)
$SSM_{1.0}$	69 (3)	62 (2)	47.11 (3.22)	92.56 (4.65)	13.11 (2.62)	14.07 (1.98)	10.96 (1.06)	13.82 (0.67)	19.88 (1.56)
$SSM_{2.5}$	68 (3)	63 (3)	42.27 (4.02)	93.18 (4.09)	10.46 (2.01)	14.36 (1.25)	11.08 (0.99)	14.01 (0.68)	16.24 (1.52)
$SSM_{5.0}$	68 (5)	60 (3)	43.15 (2.95)	95.16 (4.58)	12.32 (1.59)	14.02 (2.00)	10.56 (1.22)	13.53 (0.72)	17.62 (2.04)
$SSM_{10.0}$	67 (3)	60 (2)	39.89 (3.43)	95.18 (4.32)	10.09 (1.73)	13.87 (0.93)	10.87 (1.07)	13.86 (0.69)	15.25 (1.87)

Table 7: Nutrient composition (%) of cowpea seeds harvested from mature plants sown in a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *Pleurotus tuberregium*

	CP	CHO	CF	EE	DM	N	P	K	Ca	Mg	Na
SSM_0	20.78	64.02	6.24	7.86	88.75	3.32	0.42	1.49	0.19	0.15	0.76
$SSM_{1.0}$	21.01	63.56	6.07	8.00	88.32	3.36	0.40	1.47	0.17	0.16	0.72
$SSM_{2.5}$	20.59	64.03	5.96	7.59	87.61	3.29	0.40	1.36	0.17	0.18	0.75
$SSM_{5.0}$	20.02	65.11	6.04	7.68	88.02	3.20	0.37	1.30	0.21	0.18	0.83
$SSM_{10.0}$	18.46	65.97	5.58	7.19	87.83	2.95	0.39	1.22	0.18	0.20	0.79

No significant differences (p = 0.05) in specific nutrient composition among values

The pattern of growth of mycelia of *P. tuberregium* on WEO may be due to the fact that engine oil is just a single petroleum product and may contain chemical additives responsible for inhibiting growth. The growth of *P. tuberregium* was reduced compared with growth on higher concentrations of oil. Odjegba and Sadiq (2002) reported that engine oil usually contains chemical additives, which include amines, phenols, benzene, calcium, zinc, barium, magnesium, phosphorus, sulphur and lead. The reduction in mycelial growth as concentration of pollutants increased could be due to the toxicity of pollutants. The ability of this mushroom to tolerate the pollutants and grow on them, suggests it could be employed as bioremediation agent on sites contaminated by these pollutants.

The effects of oil pollution on the growth, development and performance of cowpea were devastating (Table 4-7). At 5 MAP, growth was adversely affected in the polluted soils, except in the control (unpolluted). All the cowpea plants in the polluted soil treatments died within two weeks, beginning first with signs of foliar chlorosis. However, following 9 months after augmentation, all plants grew up to maturity, though the effects of soil pollution was still significantly obvious as there were decreases in both yield and growth parameters along lines of increasing level of oil pollution of soil. Plant yield decreased from 26.01 g/plant in the control to 15.25-19.88 g/plant in the treated soils. Though decrease was significant in comparison to the control, effects of pollution in $SSM_{2.5}$, SSM_5, and SSM_{10} were not significantly different from one another. The implication was that of the positive effect of the treatments applied to the polluted soils.

No significant changes were recorded when nutrient composition of cowpea seeds was compared for soil treatments (Table 7). Crude protein ranged from 18.46-21.03%), total carbohydrates (62.97-67.82%), crude fibre (5.21-6.24%), total dry matter (87-61-89.26%), P (0.37-0.45%), Ca (0.17-0.21%), Na (0.72-0.83%). Fe content in seeds of cowpea was 5.6 mg/L in the control, and 7.18-22.98 mg/L in the treated soils (Table 8). However, Fe content in leaves of cowpea was 39.43 mg/L in the control, and 50.63-94.58 mg/L in the treated soils. Mn content of seeds and leaves were 0.20-0.72 mg/L and

Table 8: Heavy metal contents (mg/L) of cowpea seeds and leaves (per dry weight) harvested from mature plants sown in a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *Pleurotus tuberregium*

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
SEEDS	SSM_0	5.60	0.2	0.20	ND	ND	ND	ND	ND	ND	ND
	$SSM_{1.0}$	7.10	80.35	0.35	ND	ND	ND	ND	ND	ND	ND
	$SSM_{2.5}$	13.36	0.42	0.50	ND	ND	ND	ND	ND	ND	ND
	$SSM_{5.0}$	19.56	0.58	0.61	ND	ND	ND	ND	ND	ND	ND
	$SSM_{10.0}$	22.98	0.72	0.75	ND	ND	ND	ND	ND	ND	ND
LEAVES	SSM_0	39.43	9.98	1.06	Trace	ND	ND	ND	ND	ND	0.43
	$SSM_{1.0}$	94.58	12.18	1.79	Trace	Trace	ND	ND	ND	ND	5.59
	$SSM_{2.5}$	50.63	12.49	2.08	Trace	Trace	ND	Trace	ND	ND	10.18
	$SSM_{5.0}$	75.42	14.79	2.62	0.01	0.015	ND	0.016	ND	ND	14.96
	$SSM_{10.0}$	93.17	13.11	3.00	0.01	0.028	Trace	0.019	ND	ND	17.18

Trace Is Defined as Concentration ≤0.001 Mg/l; nd: not dermined; sensitivity of equipment being 0.0001 mg/l

Table 9: Bioaccumulation quotients for heavy metals in cowpea seeds and leaves that were harvested from mature plants sown a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with *pleurotus tuberregium*

		Fe	Mn	Zn	Cu	Cr	Pb	Cd,Ni,V,THC
Seeds	SSM_0	0.04	0.01	0.02	N/A	N/A	N/A	N/A
	$SSM_{1.0}$	0.03	0.01	0.02	N/A	N/A	N/A	N/A
	$SSM_{2.5}$	0.04	0.01	0.03	N/A	N/A	N/A	N/A
	$SSM_{5.0}$	0.04	0.02	0.04	N/A	N/A	N/A	N/A
	$SSM_{10.0}$	0.05	0.03	0.03	N/A	N/A	N/A	N/A
Leaves	SSM_0	0.25	0.41	0.12	N/A	N/A	N/A	N/A
	$SSM_{1.0}$	0.34	0.40	0.13	N/A	N/A	N/A	N/A
	$SSM_{2.5}$	0.42	0.43	0.13	N/A	N/A	N/A	N/A
	$SSM_{5.0}$	0.34	0.59	0.15	0.01	0.02	0.03	N/A
	$SSM_{10.0}$	0.39	0.51	0.12	0.01	0.03	0.04	N/A

BQ≥1: significant bioaccumulation of heavy metals occurred in leaves; N/A: not available for determination

Table 10: Hazard quotients for phytotoxicity of a 5-month old waste engine oil-polluted soil after additional nine months of bioaugmentation with Pleurotus tuberregium, to cowpea

		Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
5 MAP	SSP_0	0.04	0.46	0.02	150 *	N/D	N/D	0.083	0.93
	$SSP_{1.0}$	0.06	0.73	0.03	2.30 *	N/D	0.009	0.087	1.03 *
	$SSP_{2.5}$	0.07	0.96	0.04	2.6 *	0.005	0.016	0.107	1.06 *
	$SSP_{5.0}$	0.07	1.13 *	0.04	2.8 *	00.008	0.028	0.140	1.24 *
	$SSP_{10.0}$	0.08	1.37 *	0.04	3.8 *	0.008	0.042	0.137	1.74 *
9 MAB	SSM_0	0.06	0.21	0.007	0.32	N/D	N/D	0.002	0.03
	$SSM_{1.0}$	0.07	0.37	0.007	0.69	N/D	0.004	0.003	0.05
	$SSM_{2.5}$	0.07	0.42	0.012	0.78	0.002	0.009	0.004	0.05
	$SSM_{5.0}$	0.05	0.46	0.013	0.92	0.003	0.016	0.003	0.06
	$SSM_{10.0}$	0.06	0.62	0.014	1.08 *	0.005	0.017	0.004	0.07

*: Indication of toxicity to cowpea; HQ≥1: the implication is that there is the possibility for toxicity of heavy metal to cowpea

9.98-14.79 mg/L, respectively. Cu, Cr, Cd, Pb, Ni and V as well as THC were not determined, the sensitivity of the measuring equipment being 0.0001 mg/L. They were however present in the leaves in significant or trace amounts (<0.001 mg/L).

Bioaccumulation of heavy metals in the seeds and leaves was expressed as a quotient (BQ) to express the possibility of the contaminant being significantly accumulated in plant parts, thereby posing health threats (Table 9). But, BQ<1 for all pollutants in all the soil treatments (BQ = 0.01 to 0.05), implying that there was no significant bioaccumulation of heavy metals in cowpea seeds was recorded. Accumulation of Fe, Mn, and Zn was higher in leaves than in seeds of cowpea. Cu, Cr, Cd, Pb, Ni, and V were detected in trace amounts in some levels in leaves, and THC was only detected in leaves, not in the seeds. However, BQ values will show that bioaccumulation of heavy metals and THC in cowpea leaves was not significant ($p>0.05$).

Hazard quotient (HQ_{PhyTox}) was determined for soil phytotoxicity of heavy metal to cowpea in order to find which heavy metal was probably responsible for toxicity (Table 10). At 5 MAP, $HQ_{PhyTox} > 1$ in all levels of Cr (1.5-3.8), and in all polluted levels of V (1.03 -1.74), except the control (0.93), and in Zn ($SP_5 = 1.13$, $SP_{10} = 1.37$), thus signifying toxicity of these heavy metals to cowpea at 5 MAP. At 9 MAB, $HQ_{PhyTox} > 1$ only in higher pollution levels of Cr ($SSM_5 = 1.08$). The implication is that at 9 MAB, phytotoxicity of heavy metals to cowpea was only implicated for Cr for the levels stated above. Phytotoxicity was not implicated for the other heavy metals (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni, and V) and THC. The implication was that the death of all the cowpea plants at 5 MAP may have been caused by vanadium toxicity, as it was the only heavy metal that was positive for phytotoxicity at 5 MAP in only polluted soil levels, but showed no phytotoxicity at 9 MAB. More evidently was that the cowpea plants showed significant signs of foliar chlorosis before death. Toxicity symptoms of vanadium include chlorosis, while exerting overall negative effect on plant growth (Pratt, 1966).

Conclusively, the present study showed that significant bioremediation of the soil took place, resulting in significant lowering of hazard quotients and risk factors

that hitherto presented values of ecological concern. The study also showed that food crops like cowpea can grow in cleaned up waste engine oil polluted soil. Heavy metal accumulations in the harvestable part of cowpea were not more than benchmark accumulation provided by Efroymson *et al*. (1997). Although there were minimal accumulations in both seeds and leaves of cowpea, bioaccumulation quotients provided evidence that there bioaccumulations were not significant. The study also demonstrated the ability for cowpea to accumulate pollutants. The possibility for heavy accumulation of pollutants into harvestable cowpea parts, in non-remediated soils is not farfetched. Therefore, there is need for the verification of this assertion by further research. The accumulation of pollutants into cowpea seeds excluded the hydrocarbons. There is also need for further research to find out possible mechanisms of exclusion of hydrocarbons from cowpea seeds.

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