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Full Length Research Paper

Impact of soil amendment on phytotoxicity of a 5-month old waste engine oil polluted soil

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The present study investigated the effects of soil amendment on the remediation of waste engine oil (WEO) polluted soil, as well as the eventual phytotoxic effects of remediated amended soil on some growth parameters of cowpea. There were over 50% reductions from the original concentrations of Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni and V in soil nine months after amendment. Significant reductions in polyaromatic hydrocarbons were also achieved. Total PAH reduced from 538.59 to 1.10 mg/l in 10% w/w oil polluted soil. *Achromobacter* spp. *Clostridium* spp. *Sarcina* spp. and *Micrococcus* spp. were prevalent bacteria species found in the polluted soils, while prevalent fungi species included *Aspergillus niger, Penicillium*, Geotrichum and *Trichoderma*. The *Actinomycete nocardia* spp. was prevalent as well. Ecological risk factor initially posed by the presence of heavy metals in the unamended soil was significantly reduced to safe levels. Phytoassessment of the polluted soil was carried out just before soil was amended with sawdust, and results showed that virtually all the cowpea seedlings died within 2 weeks; only those seedlings in unpolluted soils survived. Nine months after soil was amended, all cowpea plants survived up to fruiting. The present study also showed that cowpea was able to bioaccumulate heavy metals into harvestable parts, though bioaccumulation quotients calculated showed that these accumulations were not significant.

Key words: Phytotoxicity, waste engine oil, soil amendment, polyaromatic hydrocarbons.

INTRODUCTION

The environment is increasingly exposed to changes resulting from both natural and anthropogenic sources. These changes could be drastic and as such affect the ecosystem substantially. The spill arising from disposal of waste engine oil (WEO), which itself is becoming a visible problem that needs serious attention, is not only attributed to service stations, draining oil from automobile and generator engines also account for some amounts of WEO dumped into the ecosystem (Anoliefo and Vwioko, 1995). Presently, due to the epileptic nature of public power supply in Nigeria, the use of private generating sets has increased the need for engine lubricants. Nigeria accounts for more than 87 million litres of WEO annually (Anonymous, 1985). The processes, therefore, is leading to the eventual removal of these heavy metals and hydrocarbon pollutants from the environment involving physical, chemical and biological alternatives. The most widely used physical and chemical procedures for clean-up, are not simple or favourable because they reintroduce poisonous contaminants into the environment.

In Africa, cowpea (*Vigna unguiculata*) is the most popular legume and the largest part of world production originates from this continent (Lambot, 2002). Cowpea is a food security crop in the semiarid zone of West and Central Africa (WCA) which ensures farm household subsistence food supply even in dry years. Recently, FAO (1996) estimated the world production area as 5.6 million ha, of which at least 90% is in West and Central Africa, and the annual world grain production is estimated at 2.7 million tonnes. Lambot (2002) points out that the industrial use of cowpea is facing some major constraints initially due to non existence of primary processors which is forcing food industries to process all available grains in the open market irrespective of the quality, which in general is poor, with a high percentage of physical defects mostly due to environmental pollution. The effects of remediation strategies on the phytotoxicity of

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WEO-polluted soil on cowpea, forms the basis for this study. This study therefore hopes to provide a clear understanding of the effects of remediation of WEO polluted soil by soil amendment with sawdust and culminating their effects on cowpea productivity and on certain growth parameters of the plant.

MATERIALS AND METHODS

Soil used in the present study was collected from an area measuring 50 x 50 m marked on a farmland. Top soil (0 - 10 cm), of known physicochemical property, was collected randomly from the marked plot in the morning (7.00 am) and placed on polythene sheets that were spread on an open platform and left in the sun until evening (5.00 pm) for drying.

Thereafter, 10 kg soil each was placed into 50 large perforated 25 l paint buckets with 8 perforations made with 2 mm diameter nails per bucket.

WEO was obtained as pooled 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 and a sample was taken as pooled from the jerry cans and analyzed for polyaromatic hydrocarbon composition.

Soils in each bucket were carefully poured out onto a flat platform covered with cellophane. WEO with 5 different levels of pollution: 0, 1.0, 2.5, 5.0 and 10.0% w/w WEO were poured into the measured soil and were thoroughly mixed, before taken back into each bucket. This process was repeated for every bucket. There were 10 replications per treatment.

The concentrations were obtained as follows: 0% (Control), no oil but 'clean' soil only; 1.0%, 100 g WEO in 10 kg Soil; 2.5%, 250 g WEO in 10 kg Soil; 5.0%, 500 g WEO in 10 kg Soil; 10.0%, 1 kg WEO in 10 kg Soil. For clarity, the 100 g WEO measured 135.0 ml.

The entire set up was left in an open shade for 5 months without mechanically disturbing the soil. Soil was carefully irrigated twice every week with 200 ml of water. After 5 months, soils were poured out of the buckets, broken, turned and thoroughly mixed. Three kilograms (3 kg) of soil was removed from each bucket, and replaced with 3 kg air-dried sawdust from *Brachistegia nigerica*. This was thoroughly mixed on flat platforms for each bucket before transferred back. These buckets were left until the end of observation period of 9 months. As there were 50 buckets, the layout is as follows:

Phytoassessment

After the entire period, progress of soil remediation was assessed for phytotoxicity to *V. unguiculata* cv. Kano White, using some growth and yield parameters as basis for comparison.

Soil in each bucket was turned, broken and properly mixed before being watered (200 ml) prior to sowing. Cowpea seeds were sown into each bucket at the rate of 5 seeds per hole and at a depth of 3 cm, and thinned down to 3 seedlings per bucket after seedlings had attained 2-leaf stage.

The soil buckets were weeded as they appeared. Although the plants were exposed to the prevailing weather condition (early rains of January - February 2008), water requirements by the crop were supplemented during very dry days by addition of 200 ml distilled water applied per bucket before sunset. The experiment was organized in a completely randomized design (CRD) with 5 treatments, each consisting of 10 replicates.

Bioecological statistical analyses performed at 5% probability, included one-way ANOVA, hazard quotients, bioaccumulation quotients as well as the environmental risk factor. Single factor analysis of variance was computed by using the SPSS-16 software (Statistics Package for Social Sciences).

Computation of hazard quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (COPEC). The hazards Quotient is expressed by the following equation:

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark}}$$

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 (BQ)

BQ expresses the possibility of the contaminant being significantly accumulated in plant parts, thereby posing health threats. The bioaccumulation quotient is expressed as:

$$BQ = \frac{\text{Concentration of accumulated pollutant in the accumulant}}{\text{Concentration of accumulated pollutant}}$$

Computation of environmental risk factor (ERF)

The environmental risk factor (ERF) is a pollution index employed to determine environmental risk in order to establish potential threat to aquatic organisms. It is employed following the sequential extraction of heavy metals from sediments. The environmental risk factor (ERF) is expressed by the following equation:

$$ERF = CSQV - \frac{Ci}{CSQV}$$

where CSQV is the Concentration sediment quality value (background/pre-industrial concentration), Ci is the Heavy metal concentration in the soil fractions, ERF < O = Potential ecological threat, ERF > O = No threat.

RESULTS AND DISCUSSION

Effects on soil physicochemical parameters

Physicochemical properties of the soil used for the present study is presented in Table 1. Five months after soil was impacted with WEO, it was amended with sawdust. Soil pH reading taken instantly ranged from5.46 - 5.50 (0 MAA). Nine months after amendment with sawdust (*9 MAA), there was minimal increase in pH ranging from 5.70 - 5.99 (Table 2). This increase could be

Table 1. Physical and chemical properties of soil used for the experiment.

Parameters	Unit	Soil
pH	-	5.58
EC	µs/cm	300
TOC	%	0.41
Total nitrogen		0.10
EA	meg/100 g soil	0.20
Na		10.90
K		1.65
Ca		15.60
Mg		11.30
Cl	mg/l	1666.00
P		153.00
NH_4N		25.40
NO_2		15.01
NO_3		30.75
SO_4		14.63
Clay	%	4.4
Silt		7.8
Said		87.8
Fe	mg/l	1009
Mn		17.00
Zn		30.00
Cu		3.90
Cr		2.18
Cd		N.D
Pb		0.03
Ni		3.60
V		1.36
THC		754.00

ND: Not determined.

from high metabolic activities possibly due to production of intermediate metabolites in the compost systems.

However, Alexander (1999) and Eweis et al. (1999) reported decrease in pH, attributing it to degradation of the compost and the hydrocarbons, which may have resulted in the release of acidic intermediates and final products that probably lowered pH of the mixture. The pH ranges observed in this experiment are well within the recommended range for composting organic materials (Kubota and Nakasaki, 1991; Marin et al., 2006).

Electrical conductivity (EC) of the soil increased from 210 µs/cm in SP_0 to 280 µs/cm in SP_{10} at 0 MAA. At 9 MAA however, there was significant reduction in soil EC from 250 - 148 µs/cm amounting to a 10.71 - 29.50% loss. It was also observed that reduction in soil EC was at lesser rate at higher soil/oil concentration: 10.71% reduction in SP_{10} compared to 29.52% in SP_0. The significantly lower concentration of EC in the oil-affected soils than in the control soils confirms the previous work of Osuji and Nkoye (2007). It is not likely that the released oil was directly responsible for the observed changes in EC since organic compounds like crude oil cannot conduct electrical current very well. However, it is possible that the anoxic biodegradation mechanism through direct dehydrogenation allowed the anaerobic metabolism of hydrocarbons in the presence of an electron acceptor such as nitrate ion, which may be partially responsible for the observed differences in EC.

The results also show that total organic carbon (TOC) in WEO contaminated soil was higher than in uncontaminated soil. There was significant increase in TOC of soil at 9 MAA, with values ranging from 2.60 – 3.01% compared to 0.95 – 0.99% at 0 MAA. This amounted in 172.22 – 346.15% increase during this period. This may be attributed to the high content of carbon in the oil. This could have been converted to soil organic carbon. Similar findings have been reported by Benka-Coker (1989) and Ekundayo and Obuekwe (1997).

There was general decrease in soil concentration of exchangeable bases at 9 MAA compared to values at 0 MAA. Percentage reduction in Na ranged from 63.47% in SP_{10} to 76.47% in SP_0. This implies that there was more reduction in soil concentration of Na in the control than in polluted soil treatments. A similar situation is observed in K^+. Percentage reduction in K relative to original soil concentration ranged from 51.81% in SP_{10} to 80.26% in SP_0. Ca^{2+} concentrations at 0 MAA did not differ significantly from one another, ranging from 13.91 - 14.82 meq/100 g. Minimal reduction at 9 MAA was recorded, ranging from 8.21 - 9.93 meq/100 g.

The present results oppose the findings of Amadi et al. (1993) who noted increases in the cations of soils treated with crude oil. Lehtomake and Niemela (1975) reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contaminated soil. This confirms the discovery in this research. The reduction in the concentration of NO_3-N in the contaminated site suggests that the process of nitrification might have reduced following the incidence of oil spillage. According to Odu (1972), oil degrading or hydrocarbon-utilizing microbes such as *Azobacter* spp. normally become more abundant while nitrifying bacteria such as *Nitrosomonas* spp. become reduced in number. This probably explains the relatively lower values of NO_3-N obtained for the contaminated soils.

Effects on soil heavy metal contents

At 0 MAA, Heavy metal composition of soil for Fe ranged from 768 – 1389 mg/l (Table 3), while that of Mn ranged from 18.5 – 38.7 mg/l. Zn concentration in soil slightly

Table 2. Effects of Soil amendment on physicochemical parameters of soil.

Time	Code	pH	EC	TOC	TN	EA	Na	K	Ca	Mg	Cl	P	NH4N	NO_2	NO_3	SO_4
			µS/cm	%			meq/100 g of soil						mg/l			
0 MAA	SP_0	5.50	210	0.95	0.12	0.16	10.2	0.76	14.33	9.83	1718	74.1	19.0	14.30	27.8	16.3
	$SP_{1.0}$	5.46	258	0.86	0.18	0.21	10.8	0.79	13.91	8.90	1692	69.8	18.8	12.90	27.2	16.0
	$SP_{2.5}$	5.49	271	0.91	0.21	0.18	10.2	0.85	14.10	8.72	1702	72.3	20.2	13.8	27.2	16.8
	$SP_{5.0}$	5.46	263	0.89	0.16	0.17	10.3	0.85	14.53	9.18	1769	70.1	21.6	13.60	28.0	17.5
	$SP_{10.0}$	5.50	280	0.99	0.13	0.21	9.8	0.83	14.82	9.33	1800	82.6	23.8	15.98	28.4	18.18
9 MAA	SP_0	5.89	148	2.60	0.39	0.35	2.40	0.15	9.10	4.56	38.77	1.98	1.89	3.51	11.86	0.72
		+6.51	*- 29.52*	*+ 173.68*	*+ 225.00*	*+ 118.75*	*- 76.47*	*- 80.26*	*-36.49*	*-56.61*	*- 97.72*	*- 97.33*	*- 90.05*	*- 75.45*	*- 57.34*	*- 95.50*
	$SP_{1.0}$	5.86	218	2.90	0.49	0.38	3.56	0.22	9.93	3.47	42.09	2.08	1.03	2.97	18.50	1.02
		+6.55	*- 15.50*	*+ 237.21*	*+ 172.22*	*+ 80.75*	*- 67.04*	*- 72.15*	*-28.61*	*-61.01*	*- 97.53*	*- 97.02*	*- 94.52*	*- 76.98*	*- 31.98*	*- 93.63*
	$SP_{2.5}$	5.70	220	2.93	0.63	0.36	3.63	0.21	9.40	3.84	53.09	2.71	1.86	3.01	16.35	0.83
		+3.83	*- 18.82*	*+ 221.98*	*+ 200.00*	*+100*	*- 64.41*	*- 75.29*	*- 33.33*	*- 55.96*	*- 98.87*	*- 96.25*	*- 90.79*	*- 78.19*	*- 39.98*	*- 95.06*
	$SP_{5.0}$	5.97	233	3.01	0.60	0.40	3.54	0.34	9.16	3.69	58.78	2.30	1.08	2.98	18.26	0.84
		+8.94	*- 11.41*	*+ 238.20*	*+ 275.00*	*+ 135.29*	*- 65.63*	*- 60.00*	*- 36.76*	*-59.8*	*- 96.59*	*- 96.72*	*- 91.67*	*- 78.08*	*- 34.79*	*- 95.20*
	$SP_{10.0}$	5.99	250	2.98	0.58	0.40	3.58	0.40	8.21	3.24	45.39	2.31	0.68	2.15	15.17	1.15
		+8.91	*- 10.71*	*+ 201.01*	*+ 346.15*	*+ 90.48*	*- 63.47*	*- 51.81*	*-44.60*	*- 65.27*	*- 97.45*	*- 97.20*	*- 97.14*	*- 83.39*	*- 46.58*	*- 93.67*

MAA, months after soil amendment; Italicized numbers with +ve and –ve signs represent percentage gains and losses respectively compared to values from those at 0 MAA.

reduced from 22.8 – 68.6 mg/l. Similarly, slight decreases were recorded in Cu, Ni, and V. However, no significant change in concentration of Cr and Cd was recorded. Total hydrocarbon content (THC) of soil ranged 362 – 8521 mg/l. Evidently, there was heavy metal remediation between the time soil was directly polluted with WEO and 5 months later, when experimental addition of soil amendments (sawdust) occurred. This probably was as a result decreases in heavy metal concentrations were obtained compared to those at 0 MAA.

Effects on soil polyaromatic hydrocarbon contents

Significant decreases in soil total polyaromatic hydrocarbons (TPAH) was recorded (Table 4). TPAH decreased from 130.55 mg/l to 2.23 mg/l in SP_1, 237.04 mg/l to 5.79 mg/l in $SP_{2.5}$, 358.84 mg/l to 6.35 mg/l in $SP_{5.0}$, and from 538.59 mg/l to 1.10 mg/l in $SP_{10.0}$ respectively. Also, there was total removal of PAH compounds at 9 MAA comparative to initial concentrations at 0 MAA, except for acenaphthene, acenaphthylene, anthracene, benzo(a)pyrene and phenanthrene. These PAH compounds however showed significant decreases from their original concentrations at 0 MAA. PAH reductions may have resulted

Table 3. Effects of soil amendment on heavy metal composition of soil.

Time	Code	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
						mg/l					
	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
0 MAA	$SP_{2.5}$	1063	35.6	47.8	3.8	2.6	0.02	0.80	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
	SP_0	206 - 73.12	27.9 + 50.81	10.3 - 54.82	0.82 - 64.35	0.40 - 73.33	N/D	ND	0.07 - 97.25	0.065 - 96.50	33.80 - 90.66
	$SP_{1.0}$	303 -70.84	32.3 +6.95	20.1 -44.62	1.10 -65.63	0.48 -79.13	N/D	0.17 -62.22	0.10 - 96.15	0.093 - 95.49	283.51 - 90.64
	$SP_{2.5}$	347 -67.35	34.6 - 2.81	28.6 -40.16	1.30 - 65.79	0.52 -80.00	0.007 -65.00	0.33 - 58.75	0.08 - 97.50	0.088 - 95.48	621.93 - 84.85
9 MAA	$SP_{5.0}$	618 -43.61	36.3 - 1.62	28.5 -49.38	1.37 - 62.97	0.63 -77.50	0.018 -40.00	0.62 - 56.03	0.13 - 96.90	0.102 - 95.88	838.11 - 88.04
	$SP_{10.0}$	638 -54.07	27.4 - 29.70	36.9 -46.21	1.48 - 64.76	0.75 -80.26	0.014 -53.33	0.78 - 56.43	0.15 - 96.34	0.164 - 95.20	926.63 - 89.13

Italicized numbers with +ve and –ve signs represent percentage gains and losses, respectively compared to values from those at 0 MAA.

Table 4. Effect of soil amendment on polyaromatic hydrocarbon content of soil.

PAH (mg/l)	0 MAA					9 MAA				
	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
Acenapthene	0.4989	0.8366	1.3013	1.8386	2.4235	0.4256	0	1.2904	0	0
Acenaphthylene	0	1.1164	1.8862	2.4629	3.2847	0.3816	0.3215	1.3362	0.3015	0.3215
Anthracene	0	4.4281	6.1823	8.0082	10.7625	0	1.1218	2.1527	2.2856	0
Benzo(a)anthracene	0	2.5396	2.8650	3.0625	3.5389	0	0	0	0	0
Benzo(a)pyrene	2.6025	2.4925	6.9330	11.1135	13.4380	0.7284	0	0	3.1252	0
Benzo(b)fluoranthene	0	0	0.8593	0	2.1187	0	0	0	0	0
Benezo(g,h,i)perylene	29.4638	89.1187	109.5631	78.0462	100.7342	0	0	0	0	0
Benzo(k)fluoranthene	0	18.2800	70.5652	196.7631	294.4268	0	0	0	0	0
Chrysene	0	0	0.1006	0	0.0735	0	0	0	0	0
Dibenzo(a,h)anthracene	0	0.3895	0.6789	1.0591	1.2879	0	0	0	0	0
Fluoranthene	0	0.9843	14.2890	23.0301	38.4333	0	0	0	0	0
Fluorene	0	0.2876	0.3942	0.4887	0.6623	0	0	0	0	0
Indeno(1,2,3-c,d)pyrene	0	0	0.6672	1.0816	2.0076	0	0	0.2718	0	0
Naphthalene	0.2389	0.2836	0.8369	0.3398	0.2995	0	0	0.7411	0	0
Phenanthrene	1.2828	3.4386	8.0375	11.2681	28.4367	0.2667	0.7869	0	0.6352	0.7800
Pyrene	2.8673	6.3542	11.8793	20.281	36.6667	0	0	0	0	0
Total	36.9542	130.5497	237.0390	358.8434	538.5948	1.8023	2.2302	5.7922	6.3475	1.1015

from evaporation and microbial degradation in a dissolved state (Jordan and Payne 1980; Kappeler and Wuhrmann, 1978).

Effects on prevalence of soil microorganisms

The present study recorded most prevalent bacteria

Table 5. Effect of soil amendment on distribution of soil microorganisms.

		0 MAA					5 MAA					9 MAA				
		SP_0	$SP_{1.0}$	$SP_{2.5}$	$SP_{5.0}$	$SP_{10.0}$	SP_0	$SP_{1.0}$	$SP_{2.5}$	$SP_{5.0}$	$SP_{10.0}$	SP_0	$SP_{1.0}$	$SP_{2.5}$	$SP_{5.0}$	$SP_{10.0}$
1.	*Achromobacter* spp.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
2.	*Clostridium* spp.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
3.	*C. perfringens*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
4.	*Sarcina* spp.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
5.	*Micrococcus* spp.	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
6.	*M. luteus*	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
7.	*Bacillus pumilis*	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-
8.	*B. subtilis*	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-
9.	*A. niger*	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+
10.	*A. Flavus*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	*A. fumigatus*	+		-	-	-	+	-	-	-	-	+	+	-	-	-
12.	*Penicillium* spp.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
13.	*P. notatum*	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+
14.	*Fusarium* spp.	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
15.	*Mucor* sp	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16.	*Geotrichum* spp.	+	+	+	+	+		+	+	+	+	-	-	-	-	-
17.	*Trichoderma* sp	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
18.	S*accharomyces* spp.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
19.	*Streptomyces* spp.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20.	*Nocardia* spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Bacteria: 1-8, Actinomycetes: 19-20, Fungi: 9-18. +: present, -: absent.

species as *Achromobacter*, *Clostridium*, *Sarcina* and *Micrococcus* (Table 5). Prevalent fungi were *Aspergillus niger*, *Penicillium* spp., *Geotrichum* spp., and *Trichoderma* spp. *Actinomycetes, Nocardia* sp was prevalent as well. These microorganisms may have been involved in the remediation process, considering the fact that their prevalence, even in higher concentrations of pollution, may signify tolerance to these pollutants. These identified as active members of bioremediation microbial consortia by Cerniglia (1992), Ekundayo and Obuekwe (1997), Yogambal and Karegoudar (1997), Remero et al. (2001) and April et al. (2000).

Impact of remediation on cowpea (phytoassessment)

The effects of oil pollution on the growth, development and performance of cowpea may be very devastating. Table 6 presents the germination parameters of cowpea (*V. unguiculata c*v. 'Kano White') 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 SP_{10} compared to 3.8 days in SP_0. At one week after sowing (1 WAS), percentage emergence of cowpea seedling was 82.14% in SP_0 (control), 78.57% in $SP_{1.0}$ and 28.57% in $SP_{10,}$ inferring inhibited germination rate with increased oil pollution.

At nine days after sowing (9 DAS), cowpea seedlings were 14.6 cm long in SP_0, 11.3 cm long in $SP_{1.0}$ and 7.6 cm long in $SP_{10.0}$, giving a 48% decrease in $SP_{10.0}$ in comparison with the control. Fresh dry weight at 9DAS decreased from 0.763 g in SP_0 through 0.218 g in $SP_{10.0}$.

However, during two weeks of observation, seedlings in soil of higher oil concentration began to turn yellow, until they gradually dried up. This was observed all-round from $SP_{1.0}$ - $SP_{10.0}$. Hence, at 2 WAP, seedlings in $SP_{10.0}$ had already dried out. Percentage survival was 0% in 2 WAP. Survival rate in two weeks was 82.14% in SP_0, 71.43% in $SP_{1.0}$, 57.14% in $SP_{2.5}$ and 28.57% in $SP_{5.0}$, decreasing in that order until seedlings in all polluted soil treatments finally dried up with time.

Noticeably, yellowing was first observed in the control (SP_0) at 19 DAS, but the plants recovered over time. However, yellowing in SP_1 was first observed after 11 days, 10 days in $SP_{2.5}$ and 8 and 9 days in both $SP_{5.0}$ and $SP_{10.0}$ respectively. These plants also began to gradually become necrotic from the 12^{th} day in $SP_{1.0}$, 13^{th} day in $SP_{2.5}$ and 11^{th} day in both $SP_{5.0}$ and $SP_{10.0}$ treatments respectively. Total death of the entire seedlings was recorded 7 days after yellowing in $SP_{1.0,}$ 8 days after yellowing in $SP_{2,}$ 8 days after yellowing in $SP_{3,}$ and 4 days after yellowing in $SP_{4.}$

Table 6. Germination parameters of cowpea sown at 0 MAA.

Treatments	No. of days taken for seedling emergence	Percentage emergence at 1 WAS (%)	Height of emergent in 9 DAS (cm)	Fresh wt. of emergents at 9 DAS (g)	Dry wt. of emergents at 9 DAS (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	82.14	14.6	0.763	0.249	82.14	19	0	0
$SP_{1.0}$	4.2	78.57	11.3	0.323	0.219	71.43	11	12	18
$SP_{1.6}$	4.8	64.29	9.7	0.428	0.200	57.14	10	13	18
$SP_{5.0}$	5.2	57.14	10.2	0.315	0.156	28.57	8	11	16
$SP_{10.0}$	7.6	28.57	7.6	0.218	0.117	0	9	11	13

DAS: days after sowing; WAS: weeks after sowing.

However, after soil amendment, germination was greatly improved over time (Table 7). There was no significant change in the number of days taken for initial seedling emergence, ranging from 3.6 - 4.5 days. However, percentage germination decreased from 80.00 - 42.86% according to corresponding increasing pollution levels.

Many authors (Udo and Fayemi, 1975; Anoliefo and Vwioko, 1995; Osubor and Anoliefo, 2003; Vwioko and Fashemi, 2005) have studied the effects of oil pollution on seed germination of crop plants, and all agree that oil pollution inversely affected crop germination. Oil contaminated soil generally causes delayed seed emergence and that of WEO-contaminated soil is not different. Germination of *Ricinus communis* in WEO-polluted soil was inhibited (Vwioko and Fashemi, 2005). Udo and Fayemi (1975) reported that maize germination was adversely affected by the pollution of the soil, effect being proportional to the level of crude oil pollution. At 0 MAA, seedlings in polluted soils died after 2 weeks, where as those in the unpolluted soils survived. The embryo of the seed could be killed or injured if it comes in contact with crude oil. Penetration of crude oil through seed micropylar end, scar, crack or injury would certainly endanger the life and growth of embryo, which are vital to germination. Obviously, the integrity and hardness of the seed coat affect the rate of penetration. The high content of aromatics in the oil might explain the considerate growth inhibition and subsequent death of seedling.

There were significant differences in the heights of the seedlings in the used oil polluted soils and those of the non-polluted soils. There were gross reductions in the number of leaves obtained in the seedlings of *V. unguiculata* from the polluted soils (Kayode et al., 2009). Nwoko et al. (2007) observed a reduction in chlorophyll content of the contaminated plant, indicative of the fact that our test crop grows under stress.

Results obtained from this study agreed with the previous assertion (Udo and Fayemi, 1975; Anoliefo and Vwioko, 1995; Osubor and Anoliefo, 2003; Vwioko and Fashemi, 2005) that used engine oil affect plant height, stem girth, leaf area and number of leaves in crop plants. Oil polluted soil could become unsuitable for plant growth due to a reduction in the level of available plant nutrients or a rise to a toxic level of elements (Udo and Fayemi, 1975).

No significant change in nutrient composition of cowpea seeds was observed among treatments (Table 8). Crude protein content ranged from 18.70 - 21.03%. Total carbohydrate content ranged fro 63.09 – 66.06%, where as dry matter was 87.94 – 89.07%. Values obtained for crude protein and total dry matter fall within the ranges previously recorded by Ikhajiagbe et al. (2007).

An explanation for possible heavy metal poisoning of cowpea

Of concern in the present study was the death of virtually all the cowpea seedlings (within 2 weeks) in polluted soil at 0 MAA. Only those seedlings in unpolluted soils survived. At 9 MAA however, all cowpea plants survived up to fruiting. This may have been as a result of heavy metal poisoning.

An explanation was sought for this phenomenon by trying to use ecological benchmarks and ecological quotients. When the hazard quotient for toxicity of heavy metals to cowpea was computed (Table 9), HQ was greater than unity (HQ > 1) in Cr, Zn, and V at 0 MAA, prior to soil amendment. This indicated phytotoxicity of the heavy metals. However, at 9 MAA, HQ was less than unity in

Table 7. Effect of soil amendment on some growth and yield parameters of cowpea after 9 MAA.

Germination parameters

Treatments	No. of days taken for seedling emergence	Percentage emergence at 2 WAS (%)	Height of emergents at 1 WAS (cm)	Fresh wt. of emergents at 1 WAS (g)	Dry wt. of emergents at 1 WAS (g)
SP_0	4.1	80.00	8.09	0.542	0.278
$SP_{1.0}$	3.6	70.00	6.05	0.461	0.204
$SP_{2.5}$	4.0	65.71	7.35	0.493	0.246
$SP_{5.0}$	4.5	61.43	6.93	0.466	0.237
$SP_{10.0}$	4.4	42.86	6.49	0.415	0.219

Growth parameters at 17 WAS

Treatments	Shoot height	Stem width (mm)	Leaves/plt	Leaflet area (cm^2)	No. of primary root branches/plt	Root length (cm)	No. of nodules/plt	10 nodule wt (g)	Plt dry wt. (g)	Root dry wt. (g)	S:R ratio
SP_0	103.18	9.8	18.7	66.01	7.00	50.03	20.15	1.02	12.16	0.611	18.90
$SP_{1.0}$	89.79	9.2	17.5	63.15	6.82	43.67	12.46	0.76	12.20	0.597	19.44
$SP_{2.5}$	81.08	9.2	17.0	63.61	6.09	44.19	10.23	0.76	11.18	0.500	21.36
$SP_{5.0}$	65.87	8.8	16.8	67.05	9.15	38.33	14.62	0.88	10.96	0.506	20.66
$SP_{10.0}$	75.86	8.6	15.8.	62.72	8.75	42.19	10.33	0.85	11.08	0.527	20.02

Yield parameters

Treatments	Day of prod. of 1^{st} pod (DAS)	Day of 1^{st} flowering (DAS)	No. of flowers/plt at 15WAS	Harvest day (DAS)	Pods/plt	Pod length (cm)	Seed/pod	100 seed wt. (g)	Yield/plt (g/plt)
SP_0	68	62	45.53	91.83	15.13	14.18	12.02	14.23	25.88
$SP_{1.0}$	71	61	42.11	92.56	11.56	14.23	11.11	14.02	18.01
$SP_{2.5}$	71	63	42.86	95.11	10.14	13.85	11.37	13.57	15.65
$SP_{5.0}$	69	63	44.25	95.21	9.56	12.56	10.98	13.81	14.50
$SP_{10.0}$	69	63	46.02	96.06	9.41	13.01	11.15	12.56	13.60

WAS: Weeks after sowing; DAS: Days after sowing.

Table 8. Nutrient composition (%) of seeds of cowpea.

MAP	CP	CHO	CF	EE	DM	N	P	K	Ca	Mg	Na
SP_0	21.03	63.09	5.81	8.03	89.07	3.36	0.39	1.53	0.17	0.15	0.78
$SP_{1.0}$	20.53	63.27	6.06	7.26	88.14	3.28	0.40	1.43	0.19	0.14	0.75
$SP_{2.5}$	20.67	64.11	5.62	7.17	89.26	3.31	0.41	1.38	0.17	0.17	0.73
$SP_{5.0}$	20.07	65.39	5.81	7.36	88.43	3.21	0.41	1.27	0.21	0.17	0.78
$SP_{10.0}$	18.70	66.06	5.33	7.23	87.94	2.99	0.37	1.27	0.19	0.21	0.82

CP: crude protein, CHO: total carbohydrate, EE: ether extract, DM: dry matter.

these heavy metals, indicating a non-toxic situation. Of important note is the fact that heavy metal poisoning may not be the only reason for death cowpea seedlings. Other conditions may include PAH poisoning, impacted physical condition of the soil and a host of others.

Chromium, vanadium, and zinc are not known to be essential for plant growth. However in higher soil concentrations, they may be toxic to plant. Symptoms of toxicity of chromium include stunted growth, poorly developed roots and leaf curling. Chromium may interfere with C, N, P, Fe, and Mo metabolism and enzyme reactions (Kabata-Pendias and Pendias, 1984). Toxicity symptoms of vanavium include chlorosis, dwarfing and inhibited root growth (Pratt, 1966). Vanadium inhibits

Table 9. Hazard quotients for soil phytotoxicity to cowpea.

	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
0 MAA								
SP_0	0.04	0.46	0.02	*1.5	N/D	N/D	0.083	0.93
$SP_{1.0}$	0.06	0.73	0.03	*2.3	N/D	0.009	0.087	*1.03
$SP_{2.5}$	0.07	0.96	0.04	*.2.6	0.005	0.016	0.107	*1.06
$SP_{5.0}$	0.07	*1.13	0.04	*2.8	0.008	0.028	0.140	*1.24
$SP_{10.0}$	0.08	*1.37	0.04	*3.8	0.008	0.042	0.137	*1.74
9 MAA								
SP_0	0.06	0.21	0.008	0.4	N/D	N/D	0.002	0.03
$SP_{1.0}$	0.06	0.40	0.011	0.48	N/D	0.003	0.003	0.05
$SP_{2.5}$	0.07	0.57	0.013	0.52	0.002	0.007	0.003	0.04
$SP_{5.0}$	0.07	0.57	0.014	0.63	0.004	0.012	0.004	0.05
$SP_{10.0}$	0.05	0.74	0.015	75	0.003	0.016	0.005	0.08

*Indication of toxicity to cowpea.
When HQ ≥ 1, the implication is that there is the possibility for toxicity of heavy metal to cowpea.

various enzyme systems while stimulating others, the overall effect on plant growth being negative (Peterson and Girling, 1981). After uptake, most vanadium remains in the root system in insoluble form with Ca (Wallace and Romney, 1977). Toxicity symptoms of zinc include chlorosis and depressed plant growth (Chapman, 1966). It acts to inhibit CO fixation, phloem transport of carbohydrates and alter membrane permeability (Collins, 1981).

IMPLICATIONS OF BIOACCUMULATION OF HEAVY METALS IN COWPEA

The present study has shown that cowpea was able to bioaccumulate heavy metals into harvestable parts (Table 10). Although bioaccumulation quotients calculated showed that these accumulations were not significant, these however have implications for the health and safety of consumers. Many metals act as biological poisons. The toxic elements accumulated in organic matter in soils are taken up by growing plants. The metals are not toxic as the condensed free elements but are dangerous in the form of cations and when bonded to short chains of carbon atoms. Many metals with important commercial uses are toxic and hence undesira-ble for indiscriminate release into the environment, the use of wastes in crop production since it may be possible for heavy metal from the waste to accumulate in soils and thereby enter the food chain, contaminate surface and underground water thus causing health hazard. The high risk, therefore, of exposure to heavy metal due to plant uptake of these toxic elements makes the use of polluted soils, abandoned waste dump site, irrigated soils with sewage water or any other form of polluted soils to be very risky, ensuring therefore that these soils are completely free of potential toxins.

Conclusion

For efficient bioremediation, soil amendment or additives such as sawdust, are added to increase micro-organisms activities as well as to improve the soil's physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure (Davis and Wilson, 2005). In the present study, the environmental risk factor (ERF) computations showed that Pb and V were a potential ecological risk prior to soil amendment (Table 11). Nine months after soil amendment, these heavy metals were no longer a threat. The presence of soil amendments, with its attendant microbial population, enhanced the bioremediation of WEO pollutants. The organisms, while growing on the sawdust substrate, probably produce enzymes that were used in metabolizing the hydrocarbons in the compost matrix (Diaz et al., 1996). The addition of ripe or mature compost to soil polluted with PAHs was used by Martens (1982) to remove hydrocarbons from the soil. Kastner and Mahro (1996) followed this work up by investigating the degradation of naphthalene, anthracene, fluoranthene and pyrene in soil and soil-compost incubations. The study showed that the presence of compost enhanced the removal of the PAHs. Apart from the benefits of bioremediation posited by soil amendment, it also improves the soil's physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure.

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Table 10. Heavy metal accumulation in cowpea seeds after harvest.

Heavy metal contents (mg/l) of cowpea seeds per dry weight				
MAP	**Fe**	**Mn**	**Zn**	**Cu, Cr, Cd, Pb, Ni, V, THC**
SP_0	5.16	0.20	0.19	ND
$SP_{1.0}$	7.32	0.32	0.32	ND
$SP_{2.5}$	13.11	0.40	0.53	ND
$SP_{5.0}$	18.18	0.52	0.64	ND
$SP_{10.0}$	23.46	0.70	0.76	ND
Bioaccumulation quotients (BQ) for heavy metals in cowpeas seeds				
	Fe	**Mn**	**Zn**	**Cu, Cr, Pb, Cd, Ni, V,THC**
SP_0	0.04	0.01	0.02	N/A
$SP_{1.0}$	0.03	0.01	0.02	N/A
$SP_{2.5}$	0.04	0.02	0.03	N/A
$SP_{5.0}$	0.04	0.02	0.03	N/A
$SP_{10.0}$	0.05	0.03	0.03	N/A

N/A: Not available; ND: Not detected.
When BQ ≥ 1, significant bioaccumulation of heavy metals occurred in seeds of cowpea.

Table 11. Environmental risk factor (ERF).

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
0 MAA									
SP_0	1008.23	16.94	29.24	3.31	1.9	N/D	N/D	2.91	0.86
$SP_{1.0}$	1007.97	15.22	28.79	3.08	1.12	N/D	-14.97*	2.88	0.78
$SP_{2.5}$	1007.95	14.91	28.41	2.93	0.99	N/D	-26.64*	2.71	0.72
$SP_{5.0}$	1007.91	14.83	28.13	2.95	0.90	N/D	-46.97*	2.43	0.53
$SP_{10.0}$	1007.62	14.72	27.71	2.82	0.44	N/D	-69.30*	2.46	-0.01*
9 MAA									
SP_0	1008.80	15.36	29.66	3.87	2.00	N/D	N/D	3.85	1.83
$SP_{1.0}$	1008.70	15.10	29.33	3.86	1.96	N/D	5.64	3.57	1.81
$SP_{2.5}$	1008.66	14.96	29.05	3.86	1.94	N/D	10.67	3.58	1.81
$SP_{5.0}$	1008.39	14.86	29.05	3.85	1.89	N/D	20.64	3.56	1.81
$SP_{10.0}$	1008.37	15.39	28.77	3.85	1.84	N/D	25.97	3.56	1.77

*when ERF < 0, there is potential ecological risk; ERF > 0: no potential risk. Table shows that all heavy metals did not pose any potential ecological risk after treatment.

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