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Effects of bioremediation on spent engine oil polluted soil using bio-stimulation, bio-augmentation and phytoremediation strategies

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Abstract

This study investigated the effects of bioremediation on SEO polluted soil using bio-stimulation, bio-augmentation and phytoremediation strategies in screenhouse experiment. Oil-degrading microbes were isolated and enumerated by the use of Bushnell Hass (BH) agar. The isolated oil degraders were subjected to molecular characterization and identified. The screenhouse experiment was laid out in a Completely Randomized Design consisting of three rates of pollution (0, 200 and 400 ml), four rates of compost (0, 5, 10, 20 tha^{-1}) and four levels of microbial inoculations (control, *Pseudomonas entomophila, Pseudomonas alcaligenes, Bacillus thuringiensis*). The result revealed that from the fourteen bacterial oil degraders that were isolated from SEO polluted soil, *Pseudomonas entomophila, Pseudomonas alcaligenes, Bacillus thuringiensis* had the highest overall range of oil degraders' count which were between 0.6×10^4 and 1.2×10^4 cfu. Screenhouse results demonstrated that 10 tha^{-1} of compost increased total Protein, protease and total bacteria count in soil by 243, 133 and 35% respectively; and reduced THC in root, shoot and fruit by 17, 32 and 25% as well as reduced Pb by 28, 22 and 0% respectively. Results indicated that bio-stimulation of SEO polluted soil with 10 or 20 tha^{-1} compost efficiently ameliorated pollution effect. Results also expressed that tomato has the ability to phytoremediate SEO polluted soil by accumulating heavy metals and hydrocarbons in this order; root > shoot > fruit. Also, the result showed that all the microorganisms tested bioremediated SEO polluted soil, however *B. thuringiensis* had a more significant effect on soil properties than others.

Keywords: Spent Engine Oil, Oil degraders, Compost, Tomato plant. Corresponding Author's

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1.0 Introduction

Environmental pollution with petroleum and petroleum products (a complex mixture of hydrocarbons) has been recognized as one of the most serious current problems locally and globally.

Pollution of soil by Spent Engine Oil (SEO) is rapidly increasing due to a global increase in the usage of petroleum products (Mandri and Lin, 2007). Nigeria has been reported to account for more than 87 million litres of SEO annually (Anon, 1985) and adequate attention has not been given to its disposal (Anoliefo and Vwioko, 1995). The inappropriate disposal of SEO poses an environmental hazard with global concerns (Blodgett, 2001).

The SEO is usually obtained after servicing and subsequent draining from automobile and generator engines. The concentration of PAHs in engine oil increases with time of usage and those with two and three rings accumulate rapidly in used engine oil to very high levels (Vwioko and Fashemi 2005)

It contains a mixture of different chemicals including low to high molecular weight (C_{15}-C_{21}) compounds, lubricants, additives and decomposition products and heavy metals which have been found to be harmful to the soil and human health (Duffus, 2002).

Ekundayo *et al*. (1989) reported that marked changes occur in the physical, chemical, and microbiological properties of soils contaminated with lubricant oil. Oil displaces air and water leading to anaerobic condition (Atlas, 1977).

The vast range of substrates and metabolites present in hydrocarbon impacted soils surely provides an environment for the development of a quite complex microbial community (Butier and Mason, 1997).

Bioremediation involves the use of living organisms such as green plants and micro-organisms to remove contaminants, pollutants and toxins from soil and water. These organisms eliminate, attenuate, or transform harmful substances via biological processes. It can be used to clean up an oil spill or contaminated groundwater. Bioremediation occurs naturally (even though it could be enhanced by a number of processes), thus it is widely accepted by the general public as a safe way of treating polluted soils (Adesodun and Mbagwu, 2008). The by-product of bioremediation, mainly water, CO_2 and cell biomass are harmless and are useful for plant growth.

Hydrocarbons, including PAHs, have been long recognized as substrates supporting microbial growth. A wide range of Hydrocarbon Utilizers (HCUs) found to be useful in the soil include *Pseudomonas, Rhodococcus, Mycobacterium, Bacillus, Acinetobacter, Providecin, Flavobacter, Corynebacterium, Streptococcus* (Bhattacharya *et al*., 2002). Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent, but they take longer periods of time to grow when compared to their bacterial counterparts (Prenafeta-Boldu *et al*, 2001).

Compost is the product resulting from the controlled biological decomposition of organic material that has been sanitized through the generation of heat and stabilized to the point that it is beneficial to plant growth. Compost is an organic matter resource that supports the growth of micro and macro-organisms and can serve as a bio-stimulating agent (Babalola *et al*., 2012).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables worldwide. In Nigeria, tomato is regarded as the most important vegetable after onions and pepper (Fawusi, 1978) but it tops the list of canned vegetables. Tomato has phyto-accumulation capabilities. The uptake and accumulations of the toxic heavy metals by tomato such as cadmium (Cd), zinc (Zinc), copper (Cu), chromium (Cr), mercury (Hg), and lead (Pb) and their potential effects on human health, agriculture and natural ecosystems have been analyzed in the research conducted by Adefemi and Awokonmi (2013).

Thus, the application of a combination of compost (bio-stimulation), indigenous oil degraders (bio-augmentation) and planting of tomato plants may be an effective way of remediating oil-polluted soil. Therefore, the objective of this study is to isolate, characterize and identify indigenous oil-degrading bacteria in SEO polluted soil, evaluate their degrading capabilities and their impacts on growth, yield and fruit quality. It is also to investigate the potentials of different rates of compost and assess the phyto-accumulation potential of the tomato plant.

Enrichment Coefficient (EC), Bio-concentration Factor (BCF) and Translocation Factor (TF) can be used to evaluate plant phytoremediation potential. An EC or BCF value higher than one indicates that the plant is a hyperaccumulator, whereas a value less than one is indicative of an excluder. Translocation Factor value determines plant efficiency in heavy metals translocation from the root to the shoot. A plant is considered efficient in metal translocation from root to shoot when TF is higher than one; this is due to an efficient metal transport system.

2.0 Materials and Methods

2.1 Study site

The research was carried out at the Institute for Agricultural Research and Training (IAR&T), Ibadan, the International Institute of Tropical Agriculture (IITA), Ibadan and the screen house at the College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria.

2.2 Sample collection

The soil samples used (polluted and unpolluted) were collected from the mechanic village, Sagamu, Ogun state, Nigeria. The polluted soil sample was collected randomly from three auto-mechanic workshops that had heavy spillage of SEO, with the aid of digger and an auger at the depth of 0-20 cm. They were bulked to form a composite sample, placed in a black polythene bag and transported in an ice pack. The locations had no grasses growing on them and the soil was characterized by hardened surfaces and black colouration. While the unpolluted soil sample (agricultural top soil) was collected randomly from a farm land sited within the same premises with the aid of a hoe at the depth of 0-20 cm. They were also bulked to form a composite sample and transported in clean polythene bags. Polluted soil samples were transported to IAR&T while the unpolluted soils were transported to the screenhouse of Department of Soil Science and Land Management, Federal University of Agriculture, Abeokuta to air-dry and sieved for further usage.

2.2 Physicochemical Properties of the Soil Samples

Particle size analysis was determined by using the hydrometer method described by Gee and Bauder (1986) using sodium hexametaphosphate as the dispersing agent. The coarse sand fraction was separated from the fine sand using 1 mm sieve. The result of the physical and chemical properties of the experimental soil samples analysis is as shown in Table 1.

Soil pH was determined in a 1:1 soil to water using a pH meter with glass electrode (Thomas, 1996). Total Nitrogen was determined by macro Kjedhal digestion technique by Bremner (1996), Organic carbon was determined by wet oxidation method of Walkley and Black which was modified by Nelson and Sommers (1996).

Available Phosphorus was extracted using Bray 1 method (IITA, 1997) and determined colorimetrically using the method of Murphy and Riley (1962). The exchangeable bases were extracted with 1 N ammonium acetate (NH4OAc). The sodium (Na) and potassium (K) in the extract were determined by flame photometer, whereas calcium (Ca) and magnesium (Mg) were determined using atomic absorption spectrophotometry (Mehlich, 1953). Normal potassium chloride (KCl) was used to extract for the exchangeable acidity, which was determined by titration with sodium hydroxide (NaOH).

Effective Cation Exchange Capacity was determined by summation of total exchangeable bases and exchangeable acidity (Braize, 1998; Rhoades, 1982). Heavy metals (Cr, Pb, Co and Cd) in the soil were digested with a mixture of trioxonitrate (v) acid (HNO_3) and hydrochloric acid (HCl) in ratio 3:1 v/v. The digested samples were analyzed for their metal concentrations using atomic absorption spectrophotometry (AOAC, 2005). The result of the physico-chemical properties of the experimental soil samples analysis is as shown in table1.

Isolation of Oil Degrading Bacteria from SEO polluted soil

All the glass wares used were washed, dried, and sterilized in a hot-air oven at a temperature of 160^{o}C for 1 hour. The area (bench) where the work was done was properly swabbed with cotton wool soaked in methylated spirit. The wire loop was also sterilized by flaming before and after use, using a spirit lamp.

Oil degrading bacteria were isolated using Bushnell Hass (BH) medium as the enrichment (Atlas,1995), supplemented

with 1 ml of SEO; The oil degrading bacteria in the soil were enumerated using the pour plate method. The polluted soil sample was prepared by serial dilution ranging from 10^{-1} to 10^{-10}; Isolation was carried out by plating 1 ml of serially diluted samples on the (BH) medium ($MgSO_47H_2O$ (0.2g/l), K_2HPO_4 (1.0g/l), KH_2PO_4 (1.0g/l), $FeCl_3$ (0.05g/l), NH_4NO_3 (1.0g/l), $CaCl_2$ (0.02g/l) with pH 7.2). The plates were incubated at 30^0C for 48 hours, the plates were observed, and viable cell colony counts were recorded as Colony Forming Units (cfu) at dilution factor of the sample (10^4). Fourteen oil degraders were isolated out of which ten with highest colony counts were selected for molecular characterization and identification (Table 2).

Molecular Characterization of Oil Degrading Bacteria

Strains with oil degrading ability were identified up to their species level by 16s RNA sequencing. The sequencing reaction was performed using BigDye terminator V3.1 cycle sequencing Kit containing AmpliTac DNA polymerase (Applied Biosystems, P/N: 4337457).

The sequencing reaction-mix was prepared by adding 1 µl of BigDye v3.1, 2ul of 5x sequencing buffer and 1µl of 50% DMSO. To 4µl of sequencing reaction–mix was added, 4 pico moles of primer (2µl) and sufficient amount of plasmid. The constituted reaction was denatured at 95°C for 5 minutes.

Cycling began with denaturing at 95°C for 30 seconds, annealing at 52°C for 30 seconds and extension for 4 minutes at 60°C and cycle was repeated for a total of 30 cycles in a MWG thermocycler. The reaction was then purified on sepheadex plate (Edge Biosystems) by centrifugation to remove unbound labelled and unlabelled nucelotides and salts. The purified reaction was loaded on to the 96 capillary ABI 3700 DNA analyzer and electrophoresis was carried out for 4 hours (Table 2).

The 16s RNA gene sequences obtained, was compared with the sequences from Basic Local Alignment Search Tool (BLAST) search of National Centre for Biotechnology Information (NCBI) data bases. The strains showing more than 97% 16s RNA gene sequence similarity were considered to be of the same species (Table 2).

The three most efficient oil degraders identified by biomass determination were used in the screenhouse experiment.

2.3 Experimental Procedure

Pots of 7 liters capacity were served with 5 kg unpolluted soil. Pollution was done by spiking SEO of 0, 200 and 400 ml to 5 Kg soil respectively and mixed thoroughly with the soil using hand trowel. Compost was added 2 weeks after pollution was done. The compost was mixed thoroughly with hand trowel at the rates of 0, 5, 10 and 20 t/ha to their corresponding spent oil polluted treatments respectively and watered.

Transplanting of tomato seedlings were carried out 2 weeks after compost application. The three spent oil degrading bacteria with the highest oil degrading efficiencies from biomass determination were inoculated in corresponding treated soils a week after transplanting. Microbial inoculations were the broth cultures of *Pseudomonas entomophila*, *Pseudomonas alcaligenes* and *Bacillus thuringiensis* in cfu/ml (1 ml Bacteria suspension = 1.5 x 10^5 cfu/ml) were used to inoculate the soils at transplanting.

Plant, Fruit and Soil Sampling

Soil samples from each pot were taken at 10 Weeks After Transplanting (WAT). At 12 WAT, the mature tomato plants were uprooted carefully. The plant samples were washed with distilled water; and sorted into shoots, roots, and fruits. Collected samples were put in sampling bags and labeled appropriately.

2.4 Analyses of Plant and Soil Samples

The following plant and soil parameters were analyzed; total protein (Bradford, 1976), protease (Parry *et al*., 2001), bacteria count, total petroleum hydrocarbon (Adesodun and Mbagwu, 2008) and lead (Dewis and Freitas,1976).

Determination of Total Protein

The sample (0.2 g) was weighed into a 75 ml capacity digestion tube and a tablet of selenium catalyst was added. About 4 ml of concentrated H_2SO_4 was added followed by the addition of 4 ml of H_2O_2 (to prevent frothing). It was then placed inside a digester and left to digest. After digestion, it was frothing made up with distilled water up to 75 ml after which 1 ml was pipetted from this solution into a clean test-tube and 3 drops of mineral stabilizer was added. (This is to stabilize the mineral content in the sample). Three drops of polyvinyl alcohol was then added and made up with distilled water to 25 ml. Thereafter, 1 ml of nesseler reagent was added and left for 5 minutes for colour development. It was then read on a spectrophotometer at 460nM wavelength.

Calculation: (For Dry sample)

% N = (0.0075 X A)/ B X C

Where A = Absorbance from Spectrophotometer, B = Weight of the sample, C = ml of digest analysed (i.e 1 ml).

For Liquid Sample

(Mg/L) N = (75 X A)/ B X C; Where A = Mg/L reading, B = ml of sample digested, C = ml of digestion analysed.

Soil Protease Assay

Moist, sieved soil (2 mm) was weighed (1 g) and placed in a centrifuge tube, 5 ml Tris buffer and 5 ml sodium caseinate solution were added. The tubes were stopped, contents mixed and incubated for 2 hours at 500C on a shaking water bath. At the end of incubation, 5 ml of TCA (Trichloroacetic acid) solution was added, and the contents mixed thoroughly. For the controls, 5 ml of Na caseinate solution was added at the end of the incubation and immediately before adding the TCA solution. The resulting soil suspensions was centrifuged (10000 -12000 rev min-110min), 5 ml of the clear supernatant was pipetted into tubes, mixed with 7.5 ml of the alkaline reagent, and incubated for 15 min at room temperature. Thereafter, 5 ml of the folin reagent was added, the mixtures were filtered through paper filter into glass tubes and the absorbance measured after exactly 1 hour at 700 nm (measure the absorbance several times until the measure value becomes constant).

Calibration curve

About 0, 1, 2, 3, 4 and 5 ml of the tyrosine solution were pipetted into glass tubes and 5 ml Na caseinate was then added. The resultant mixture was brought up to 10 ml with Tris buffer followed by the addition of 5 ml TCA solution. The measurements were performed as described above.

Calculation

The measured absorbance for the controls are corrected and calculated as follows:

Protease activity (µg tyrosine g-1 dwt2h-1) = C x 15/ dwt

Where dwt is the dry weight of 1g of moist soil, 15 is the final volume of solutions added to the soil in the assay and C is the measured tyrosine concentration (µg ml-1 supernatant or filtrate).

Total Petroleum Hydrocarbon Determination

Soil sample (10 g) was weighed into a sampling bottle, 50 ml of carbon tetrachloride was added and mixed well; the mixture was separated using a separating funnel. After drying the extract, 1g Anhydrous Sodium Sulphate was added and filtered with Whatman No. 1 filter paper while Carbon tetrachloride (CCl_4) was allowed to evaporate at room temperature in a fume cupboard. The absorbance of the filtrate was read at 410 nm on the spectrophotometer. The weight of

the petroleum hydrocarbon was determined from a previously prepared standard curve.
The amount of Petroleum Hydrocarbon (PHC) degraded was calculated by subtracting the weight of the residual hydrocarbon from the weight of the added (initial) petroleum hydrocarbon divided by the weight of the initial hydrocarbon and multiply by 100.
Initial PHC = x
Residual PHC = y
Amount of PHC degraded= (x-y)/(x) x 100
Heavy Metal (Pb) Content Determination
Soil sample (2 g) was weighed into a digestion tube. One tablet of selenium catalyst was placed inside the tube. 10 ml of concentrated perchloric acid ($HClO_4$) and 10 ml concentrated nitric acid- HNO_3 (i.e ratio 1:1) were added. The tube was placed inside a digestion block, and slowly digested. The digest was washed into 100 ml volume flask and made up with distilled water. The washed sample was then read from Atomic Absorption Spectrophotometer (AAS) using the heavy metals respective lambs and wavelengths.
Calculation was done using this formula: Meter Reading X Slope X Dilution factor (Dewis and Freitas, 1976).
The lamp and wavelengths of Pb, Cd, Co and Cr were 283.3 nM, 228.8 nM, 345.4 nM and 357.9 nM respectively.
Experimental Design
The experimental design was a 3 x 4 x 4 factorial in Completely Randomized Design (CRD) with three (3) replicates resulting in 144 pots. The design consists of:
3 pollution rates of spent oil (0, 200 and 400 ml)
4 compost rates (0, 5, 10 and 20 t/ha)
4 Microbial treatments (No microorganism, *Pseudomonas entomophila*, *Pseudomonas alcaligenes* and *Bacillus thuringiensis*).
Statistical Analysis
Data collected in the screenhouse study were subjected to Analysis of Variance (ANOVA) using General Linear Model (GLM) Procedure and significant means were separated by Duncan's Multiple Range Test at $p \leq 0.05$ (DMRT).

3.0 Results

The pH of the polluted soil was acidic (pH 5), while the unpolluted soil was slightly acidic (pH 6.5). Textural class of the polluted soil is silty loam while the unpolluted soil is silt. The base saturation for both unpolluted and polluted soils were high; the organic carbon of the polluted soil was much higher than that of the unpolluted soil. However, the exchangeable bases and total nitrogen were low for both soils. Heavy metals contents were high in polluted soil but low in unpolluted soil (Table 1).
Table 1 also shows the nutrient composition of the compost. The percentage organic carbon (% C) was 41% indicating that the organic matter is high. The percentage composition of the macronutrients N, P, K, Na, and Ca were 5.82, 6.92, 5.94, 3.71 and 3.61% respectively. However, the values of Zn, Fe, Mn, Cu, Pb, Cd, Cr and As were 4.11, 3.47, 0.1, 0.2, 0.02, 0.02, 0.001 and 0.001 $mgkg^{-1}$ respectively.
Isolation, Colony Count and Molecular Characterisation
Fourteen bacterial oil degraders were isolated and selected from the SEO polluted soil. The overall range of oil degraders' count was between 0.3×10^4 and 1.2×10^4 cfu, as shown in Table 2. Isolates 1 to 10 had higher ranged between 0.4×10^4 and 1.2×10^4 cfu counts compared to that of isolates 11 to 14. This may be due to species variation.
Figure 1 showed that 16S rDNA fingerprinting were amplified, eluted, and sequenced, regarding molecular features for the ten (10) obtained hydrocarbon-degrading isolates. Strain G1 was identified as *Pseudomonas guariconensis* with 99% of homology percentage and G2 strain was blank with no identification, strain G3 was identified as *Pseudomonas aeruginosa* with 96% of homology percentage, strain G4 was identified as *Pseudomonas putida* with 96% of homology percentage, strain G5 was identified as *Pseudomonas entomophila* with 96% of homology percentage, strain G6 was identified as *Bacillus thuringiensis* with 96% of homology percentage, strain G7 was identified as *Pseudomonas alcaligenes* with 100% of homology percentage, strain G8 was identified as *Pseudomonas monteilii* with 99% of homology percentage. The nineth isolate, G9 was identified as *Pseudomonas putida* with 98% of homology percentage, strain G10 was identified as *Staphylococcus aureus* with 93% of homology percentage (Table 2).

Percentage Composition of Nitrogen, Carbon, Phosphorus and Residual Hydrocarbon
Broth inoculation of isolates revealed that *P. entomophila, B. thuringiensis* and *P. alcaligenes* produced higher nitrogen, carbon, phosphorus and lower residual hydrocarbon content than *P.guariconensis*, *P. putida, P. aureginosa, P. monteilii* and *Staphyloccus aureus*. Therefore, they were regarded as efficient oil degraders (Table 3).
Effects of Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%), Enzymes Activities-Protease (mg/kg) and Total Bacteria Count (cfu 10^5)
Table 4 revealed that at 400 ml pollution, soil contents of protein and protease (enzyme activities) were significantly lowered while TBC was considerably increased. All compost amendments (5, 10 and 20 t/ha) significantly increased total protein, protease and TBC. Similarly, microbial treatments with either *Pseudomonas alcaligenes* or *B. thuringiensis* significantly increased total protein, protease and TBC. Effects of interaction were mostly highly significant ($P \leq 0.05$) (Table 4).

Interactive effects of Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%), Enzymes Activities-Protease (mg/kg) and Total Bacteria Count (cfu 10^5) in 200 ml Polluted Soil
Figures 2, 3 and 4 revealed that at 200 ml pollution, 20 t/ha and microbial inoculation with *B. thuringiensis* or *P alcaligenes* significantly increased soil contents of protein, protease and TBC. These soil parameters were at lowest at no compost amendments.
Interactive effects of Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%), Enzymes Activities-Protease (mg/kg) and Total Bacteria Count (cfu 10^5) in 400 ml polluted Soil
Table 5 revealed that at 400 ml, 20 t/ha and microbial inoculation with *B. thuringiensis* or *P alcaligenes* significantly increased soil contents of protein, protease and TBC. These soil parameters were at lowest at no compost amendments.

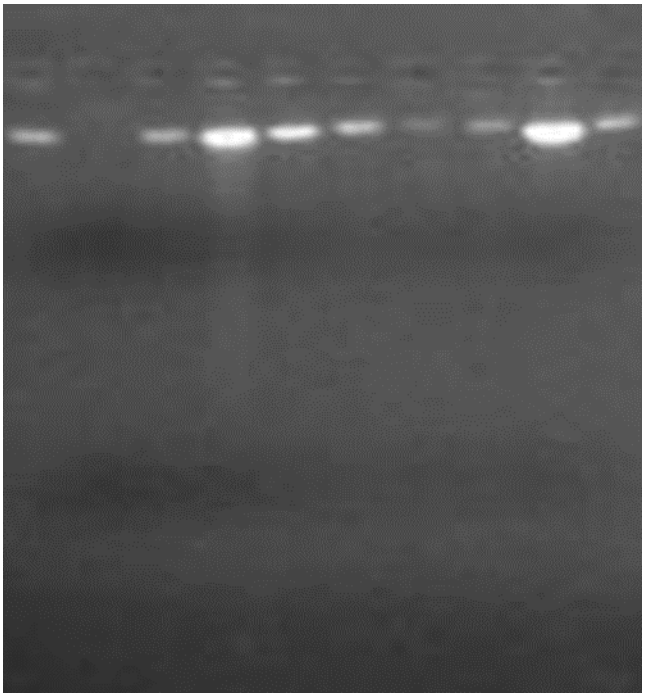
Effects of Pollution, Compost and Microbial Inoculation on Total Hydrocarbon (mg/kg) and Lead (mg/kg) of Soil, Tomato Root, Shoot, Fruit and Fruit Weight (g/plant).
Table 6 revealed that values of THC and Pb in soil, tomato root, shoot and fruit were considerably

Table 1: Physical and Chemical Properties of Top-Soil and Composition of the Compost

Parameters measured	Unpolluted Soil	Polluted Soil	Compost
Particle sizes			
% Sand	14.29	24.36	
% Silt	83.00	69.16	
% Clay	2.71	6.46	
Textural Class	Silt	Silty loam	
pH	6.50	5.60	
% organic carbon	12.75	22.16	41.00
%N	0.08	0.64	.82
Av. P (mg kg^{-1})	6.83	16.88	6.92 (%)
Exchangeable bases (cmol kg^{-1})			
Na	0.31	0.67	3.71 (%)
K	0.42	0.76	5.94 (%)
Ca	0.26	0.46	3.61 (%)
Mg	0.18	1.72	6.92 (%)
Exchangeable acidity (cmol kg^{-1})			
H^+	0.09	0.06	
Cation Exchange Capacity (CEC)	1.26	3.67	
% Base saturation	92.9	98.4	
Heavy metals (mgkg^{-1})			
Fe	0.06	2.72	3.47
Zn	0.11	6.76	4.11
Mn	0.04	8.63	0.10
Pb	0.01	14.81	0.02
Cd	0.02	12.85	0.02
Co	ND	5.43	
Cr	ND	5.72	
Hg	ND	ND	
Cu			0.20
As			0.00

Figure 1: Electrophoresis

G1 G2 G3 G4 G5 G6 G7 G8 G9 G10



Amplified product of 16S rDNA for ten bacterial isolates (G1), (G2), (G3), (G4), (G5), (G6), (G7), (G8), (G9) and (G10)

Table 2: Colony Count of Oil degraders and 16S rDNA sequencing data of the isolated strains

Isolates	Colony Count (cfu10^4)	Total length (bp)	Gene bank accession no.	Molecular Identification	Identity %
Isolate 1	0.8	1236	KX364073.1	*Pseudomonas guariconensis*	99
Isolate 2	0.4	Blank	Blank	Blank	Blank
Isolate 3	0.5	1467	KY750725.1	*Pseudomonas aeruginosa*	96
Isolate 4	0.4	979	MF111508.1	*Pseudomonas putida*	96
Isolate 5	1.2	1502	KX008299.1	*Pseudomonas entomophila*	96
Isolate 6	0.6	923	LT844652.1	*Bacillus thuringiensis*	96
Isolate 7	1.0	561	JQ246800.1	*Pseudomonas alcaligenes*	100
Isolate 8	0.6	1539	KX785170.1	*Pseudomonas monteilii*	99
Isolate 9	0.7	1230	CP018629.1	*Staphyloccus aureus*	93
Isolate 10	0.6	974	MF111953.1	*Pseudomonas putida*	98
Isolate 11	0.4	-	-	-	-
Isolate 12	0.3	-	-	-	-
Isolate 13	0.3	-	-	-	-
Isolate 14	0.4	-	-	-	-

Table 3: Percentage Composition of Nitrogen, Carbon, Phosphorus and Residual Hydrocarbon in SEO

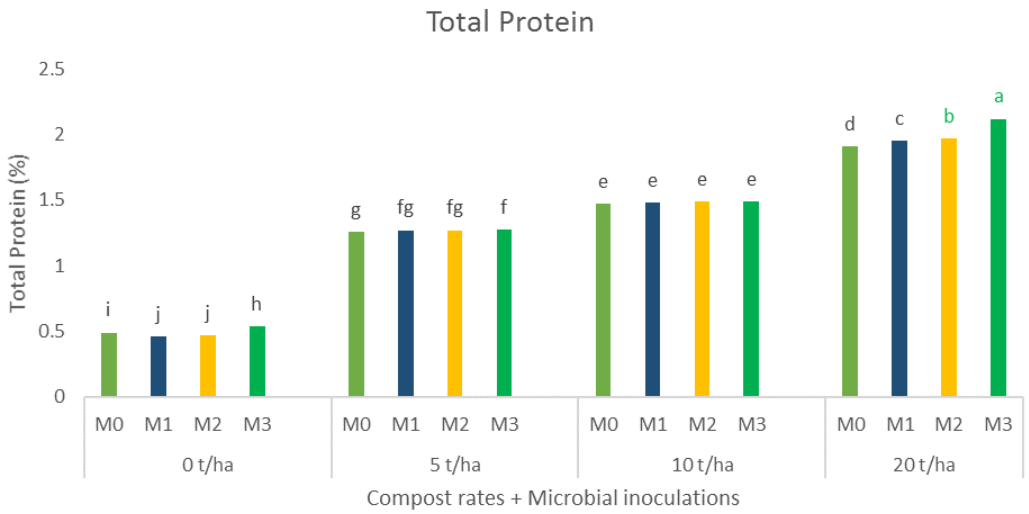
Microbes	N (%)	C (%)	P (%)	Residual Hydrocarbon (%)
P.guariconensis	0.56	61.28	0.66	48.84
P. aureginosa	0.29	34.23	0.48	67.67
P. putida	0.26	47.6	0.51	51.52
P. entomophila	0.53	63.43	0.76	40.82
B. thuringiensis	0.45	52.68	0.62	48.55
P. alcaligenes	0.63	61.36	0.71	38.80
P. monteilii	0.17	28.03	0.44	72.86
Staphyloccus aureus	0.14	26.41	0.42	73.84
P. putida	0.37	56.38	0.47	48.38
N –Nitrogen, C –Carbon and P -Phosphorus				

N –Nitrogen, C –Carbon and P -Phosphorus

Table 4: Effects of Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%), Enzymes Activities-Protease (mg/kg) and Total Bacteria Count (cfu 10^5)

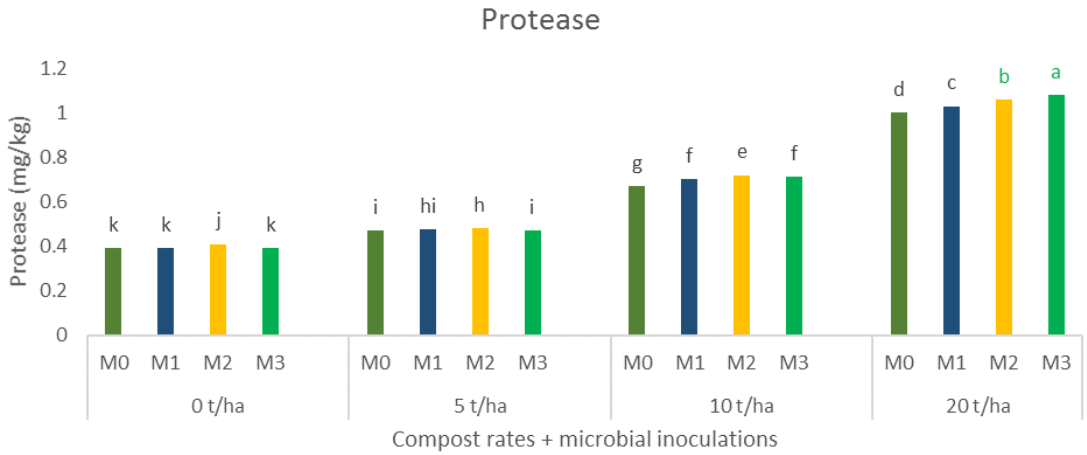
Treatments	TP	Protease	TBC
Pollution			
0 ml	1.5400 a	0.92 a	2.18 c
200 ml	1.3100 b	0.65 b	4.43 b
400 ml	1.1600 c	0.29 c	7.54 a
Compost			
0 t/ha	0.4200 d	0.30 d	3.76 d
5 t/ha	1.2100 c	0.48 c	4.36 c
10 t/ha	1.4400 b	0.70 b	5.07 b
20 t/ha	2.2700 a	1.00 a	5.66 a
Microbes			
No Microbe	1.3195 c	0.59 d	4.53 d
P. entomophila	1.3171 c	0.61 c	4.67 c
P alcaligenes	1.3240 b	0.65 a	4.77 b
B. thuringiensis	1.3700 a	0.64 b	4.88 a
P*C	**	**	**
P*M	ns	**	**
C*M	**	**	**
P*C*M	**	**	**

Values with the same letter along the column and treatments are not significantly different, Blank = not significantly, * =significant, ** =highly significant. TP -Total Protein, THC -Total Petroleum Hydrocarbon Content, TBC -Total Bacteria Count, P*C - Pollution * Compost, P * M - Pollution * Microbes, C * M - Compost * Microbes, P * C * M - Pollution * Compost * Microbes



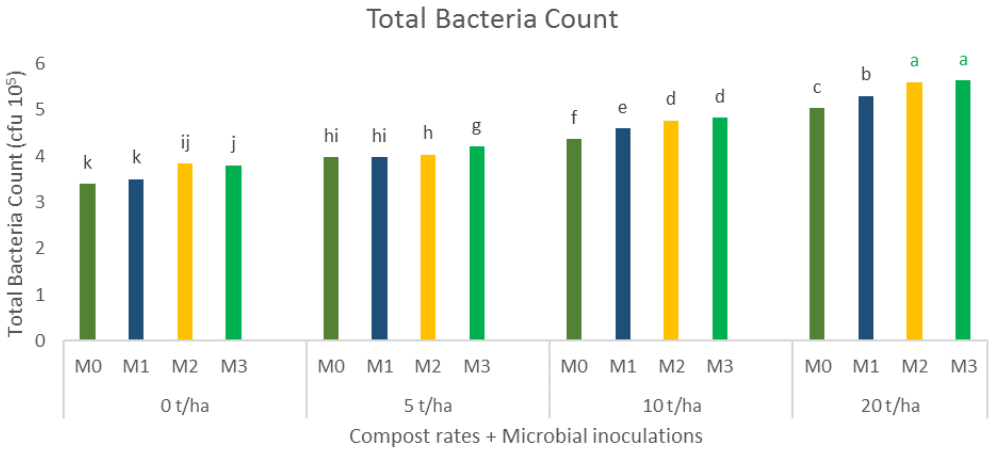
Key: M0 – No microbe, M1 – Pseudomonas entomophila, M2 - Pseudomonas alcaligenes, M3 - Bacillus thuringiensis

Figure 2: Interactive Effects of 200 ml Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%)



Key: M0 – No microbe, M1 – Pseudomonas entomophila, M2 - Pseudomonas alcaligenes, M3 - Bacillus thuringiensis

Figure 3: Interactive Effects of 200 ml Pollution, Compost and Microbial Inoculation on Enzymes Activities-protease (Mg/Kg)



Key: M0 – No microbe, M1 – Pseudomonas entomophila, M2 - Pseudomonas alcaligenes, M3 - Bacillus thuringiensis

Figure 4: Interactive Effects of 200 ml Pollution, Compost and Microbial Inoculation on Total Bacteria Count (cfu10^5)

Table 5: Interactive Effects of Pollution, Compost and Microbial Inoculation on Soil Contents of Total Protein (%), Enzymes Activities-Protease (mg/kg) and Total Bacteria Count (cfu 10^5) in 400 ml Polluted Soil

Treatments	TP	Protease	TBC
Microbes			
0 t/ha			
No Microbe	0.340 i	0.02 k	6.37 i
P. entomophila	0.310 j	0.03 k	6.37 i
P alcaligenes	0.320 j	0.04 j	6.50 i
B. thuringiensis	0.390 h	0.02 k	6.67 h
5 t/ha			
No Microbe	1.110 g	0.10 i	6.83 g
P. entomophila	1.120 fg	0.11 hi	6.97 g
P alcaligenes	1.120 fg	0.12 h	6.83 g
B. thuringiensis	1.124 f	0.10 i	7.30 f
10 t/ha			
No Microbe	1.320 e	0.31 g	7.80 e
P. entomophila	1.330 e	0.33 f	7.97 d
P alcaligenes	1.340 e	0.36 e	7.87 de
B. thuringiensis	1.340 e	0.34 f	8.23 c
20 t/ha			
No Microbe	1.760 d	0.64 d	8.53 b
P. entomophila	1.800 c	0.66 c	8.67 b
P alcaligenes	1.820 b	0.70 b	8.63 b
B. thuringiensis	1.970 a	0.71 a	9.03 a

Values with the same letter along the column and treatments are not significantly different. TP -Total Protein, THC -Total Petroleum Hydrocarbon Content, TBC -Total Bacteria Count

Table 6: Effects of Pollution, Compost and Microbial Inoculation on Total Hydrocarbon (mg/kg) and Lead (mg/kg) of Soil, Tomato Root, Shoot, Fruit and Fruit Weight (g/plant).

	Total Hydrocarbon Content				Lead				
Treatments	Soil	Root	Shoot	Fruit	Soil	Root	Shoot	Fruit	FW
Pollution									
0 ml	10 c	5.0 c	2.0 c	0.00 c	0.070 c	0.0040 c	0.00060 c	0.000 b	97.88 a
200 ml	120 b	54.0 b	5.0 b	2.20 b	2.170 b	0.0390 b	0.00400 b	0.001 a	99.52 a
400 ml	240 a	60.0 a	5.1 a	3.70 a	7.480 a	0.0450 a	0.00480 a	0.001 a	93.34 a
Compost									
0 t/ha	230 a	64.0 a	5.0 a	2.40 a	4.130 a	0.0360 a	0.00360 a	0.000 a	60.85 b
5 t/ha	130 b	60.0 b	4.3 b	2.10 b	3.500 b	0.0330 b	0.00320 b	0.000 a	98.57 a
10 t/ha	70 c	53.0 c	3.4 c	1.80 c	2.980 c	0.0260 c	0.00280 c	0.000 a	120.14 a
20 t/ha	65 d	46.0 d	2.7 d	1.50 d	2.350 d	0.0210 d	0.00240 d	0.000 a	108.09 a
Microbes									
No microbe	129 a	57.0 a	4.0 a	2.00 a	3.290 a	0.0300 a	0.00320 a	0.000 a	109.03 a
P. entomophila	126 ab	56.0 b	3.7 c	1.96 b	3.231 b	0.0290 b	0.00300 b	0.000 a	100.46 a
P.alcaligenes	124 b	56.0 b	3.6 d	1.89 c	3.230 bc	0.0286 bc	0.00292 c	0.000 a	86.47 a
B. thuringiensis	120 c	55.0 c	3.8 b	1.88 c	3.220 c	0.0283 c	0.00292 c	0.000 a	91.70 a
P* C	**	**	**	**	**	**	**		
P *M	**		**	**	**	**	**		
C *M	**		**	**	**	**	**		*
P *C *M	**		**	**	**	**	**		*

*Values with the same alphabet along the column and treatments are not significantly different, Blank = not significantly, * =significant, ** =highly significant.* P *C - Pollution * Compost, P * M - Pollution * Microbes, C * M - Compost * Microbes, P * C * M - Pollution * Compost * Microbes

Table 7: Interactive effects of Microbial Inoculation and Compost on Total Hydrocarbon (mg/kg) and Lead (mg/kg) of Soil, Tomato Root, Shoot and Fruit and Fruit Weight (g/plant) in 200ml polluted Soil.

	Total Hydrocarbon Content				**Lead**				
Treatments	Soil	Root	Shoot	Fruit	Soil	Root	Shoot	Fruit	FW
Microbes									
0 t/ha									
No microbe	310 a	60.0 b	6.3 a	2.5 a	3.11 a	0.0443 b	0.0045 a	0.00 a	93.00 bc
P. entomophila	270 b	60.0 b	6.3 a	2.5 a	2.94 b	0.0450 b	0.0043 b	0.00 a	103.10 a-c
P. alcaligenes	250 b	70.0 a	6.3 a	2.5 a	2.97 b	0.0483 a	0.0040 c	0.00 a	60.00 bc
B. thuringiensis	250 b	70.0 a	6.3 a	2.5 a	2.94 b	0.0487 a	0.0040 c	0.00 a	40.20 c
5 t/ha									
No microbe	190 c	59.5 c	5.8 b	2.5 a	2.14 d	0.0423 c	0.0038 c	0.00 a	101.60 bc
P. entomophila	190 c	59.5 c	5.8 b	2.0 b	2.24 c	0.0423 c	0.0035 d	0.00 a	78.80 bc
P. alcaligenes	190 c	59.9 c	5.7 bc	2.0 b	2.24 c	0.0423 c	0.0035 d	0.00 a	77.50 bc
B. thuringiensis	190 c	59.2 c	5.6 c	2.0 b	2.24 c	0.0420 c	0.0035 d	0.00 a	190.70 a
10 t/ha									
No microbe	100 d	52.9 d	4.0 d	2.0 b	2.13 d	0.0373 d	0.0035 d	0.00 a	77.75 bc
P. entomophila	100 d	51.2 d	3.2 f	2.0 b	2.26 c	0.0360 de	0.0035d	0.00 a	123.20 a-c
P. alcaligenes	100 d	48.5 e	3.2 f	2.0 b	2.26 c	0.0343 ef	0.0035d	0.00 a	114.37 a-c
B. thuringiensis	100 d	46.9 ef	3.2 f	2.0 b	2.26 c	0.0333 fg	0.0035d	0.00 a	67.85 bc
20 t/ha									
No microbe	100 d	45.2 f	3.8 e	2.0 b	1.26 e	0.0320 g	0.0030 e	0.00 a	124.25 a-c
P. entomophila	100 d	42.9 g	1.9 g	2.0 b	1.26 e	0.0300 h	0.0030 e	0.00 a	81.10 bc
P. alcaligenes	100 d	41.9 g	1.9 g	2.0 b	1.26 e	0.0293 h	0.0030 e	0.00 a	140.03 ab
B. thuringiensis	100 d	40.5 g	1.9 g	2.0 b	1.26 e	0.0405 g	0.0030 e	0.00 a	118.81 a-c

Values with the same alphabet along the column and treatments are not significantly different.

Table 8: Interactive effects of Microbial Inoculation and Compost on Total Hydrocarbon (mg/kg) and Lead (mg/kg) of Soil, Tomato Root, Shoot and Fruit and Fruit Weight (g/plant) in 400ml polluted Soil.

	Total Hydrocarbon Content				**Lead**				
Treatments	Soil	Root	Shoot	Fruit	Soil	Root	Shoot	Fruit	FW
Microbes									
0 t/ha									
No microbe	400 a	80.20 a	6.7 a	4.5 b	9.46 a	0.05700 a	0.0055 a	0.00 a	37.59 c
P. entomophila	400 a	77.70 b	5.5 b	5.0 a	9.26 b	0.05533 b	0.0055 a	0.00 a	35.60 c
P. alcaligenes	400 a	75.73 c	5.3 c	4.5 b	9.28 b	0.05333 c	0.0055 a	0.00 a	49.80 bc
B. thuringiensis	400 a	75.73 c	5.3 c	4.5 b	9.26 b	0.05333 c	0.0055 a	0.00 a	57.50 bc
5 t/ha									
No microbe	200 b	71.97 d	5.6 b	4.5 b	8.36 c	0.05100 d	0.0055 a	0.00 a	98.25 a-c
P. entomophila	200 b	71.80 d	5.2 d	4.0 c	8.16 d	0.05100 d	0.0050 b	0.00 a	88.18 a-c
P. alcaligenes	200 b	69.07 e	5.2 d	4.0 c	8.16 d	0.04900 e	0.0050 b	0.00 a	64.20 bc
B. thuringiensis	200 b	68.40 e	5.2 d	4.0 c	8.16 d	0.04833 e	0.0050 b	0.00 a	111.10 a-c
10 t/ha									
No microbe	200 b	62.07 f	5.0 e	3.5 d	6.82 e	0.04367 f	0.0045 c	0.00 a	120.53 a-c
P. entomophila	200 b	59.40 g	5.0 e	3.5 d	6.58 f	0.04200 g	0.0045 c	0.00 a	168.00 a
P. alcaligenes	200 b	59.07 g	4.8 f	3.2 e	6.62 f	0.04167 g	0.0045 c	0.00 a	126.71 a-c
B. thuringiensis	200 b	57.40 h	4.7 g	3.0 f	6.64 f	0.04100 g	0.0045 c	0.00 a	139.33 ab
20 t/ha									
No microbe	200 b	44.90 i	4.4 h	3.0 f	5.86 g	0.03133 h	0.0045 c	0.00 a	113.00 a-c
P. entomophila	200 b	44.90 i	3.4 i	2.5 g	5.82 g	0.03167 h	0.0045 c	0.00 a	100.07 a-c
P. alcaligenes	200 b	45.87 i	3.3 i	2.5 g	5.67 h	0.03233 h	0.0040 d	0.00 a	95.00 a-c
B. thuringiensis	100 c	44.87 i	3.4 i	2.5 g	5.62 h	0.03167 h	0.0040 d	0.00 a	88.60 a-c

Values with the same alphabet along the column and treatments are not significantly different

Table 9: Translocation Factor, Enrichment Coefficient and Bio-concentration Factor of Pb (mg/kg) in Tomato Plant Grown in Soil Amended with Compost and Microorganisms in SEO polluted Soil

Treatments	TF	EC	BCF
Microbes			
0 t/ha			
No microbe	0.096	0.001	0.007
P. entomophila	0.099	0.001	0.007
P. alcaligenes	0.103	0.001	0.006
B. thuringiensis	0.103	0.001	0.006
5 t/ha			
No microbe	0.108	0.001	0.007
P. entomophila	0.098	0.001	0.007
P. alcaligenes	0.102	0.001	0.007
B. thuringiensis	0.103	0.001	0.007
10 t/ha			
No microbe	0.103	0.001	0.007
P. entomophila	0.107	0.001	0.007
P. alcaligenes	0.108	0.001	0.007
B. thuringiensis	0.110	0.001	0.007
20 t/ha			
No microbe	0.144	0.001	0.006
P. entomophila	0.142	0.001	0.006
P. alcaligenes	0.124	0.001	0.006
B. thuringiensis	0.126	0.001	0.006

TF = Translocation Factor. TF is the ratio of the concentration of heavy metals in the shoot to the concentration of heavy metals in the root. EC = Enrichment Coefficient. EC is the ratio of the concentration of heavy metals in the shoot to the concentration of heavy metals in soil. BCF= Bio-concentration Factor. BCF is the ratio of the concentration of heavy metals in plant parts to the concentration of heavy metals in polluted soil

4.0 Discussion

Soils used in all the stages of the study were obtained from polluted and unpolluted lands with different textures, pH, organic carbon, total nitrogen, available phosphorus, exchangeable bases, exchangeable acidity, base saturation and heavy metal content. The textural class of the polluted soil was silty loam while the unpolluted soil was silt. The pH of the polluted soil was acidic while the unpolluted soil was slightly acidic. Nwaoguikpe (2011) also reported reduced soil pH in polluted soils. The organic carbon contents in polluted soils were higher than those of the unpolluted. This was attributed to the continuous deposition of SEO to the soil. Osuji and Nwoye (2007) reported reduced soil pH, increased soil organic carbon and organic matter in polluted soils. The Spent Engine Oil (SEO) polluted soils contained contaminants that added to macronutrient contents initially present in the soil before contamination. Fourteen (14) bacteria oil-degraders were encountered. Out of which, ten isolates had higher colony counts.

However molecular characterisation revealed that the ten (10) isolates were distinctly eight in number where one is void and the other was a replicate of already existing isolate, hence they were identified as *P.guariconensis, P. aureginosa, P. putida, P. entomophila, B. thuringiensis, P. alcaligenes, P. monteilii* and *Staphylococcus aureus*. Among the distinctly eight oil degraders screened, microbial biomass Nitrogen, Carbon and Phosphorus (NCP) determination by chloroform fumigation-incubation technique (Anderson and Domsch, 1978) confirmed by residual hydrocarbon determination by gravimetric analysis revealed that *P. entomophila, B. thuringiensis* and *P. alcaligenes* were the most efficient as they produced higher nitrogen, carbon, phosphorus and lower residual hydrocarbon contents. This result was supported by the work of Adebusoye *et al.* (2007) as he affirmed that *Pseudomonas sp., Bacillus sp.* and *Alcaligenes sp*. could degrade oil. Thus, *P. entomophila, B. thuringiensis,* and *P. alcaligenes* were further used for screenhouse study.

Pollution rates (200 and 400 ml) reduced soil Total Protein (TP) and protease, while concentrations of Total Hydrocarbon Content (THC) and Pb, were increased compared to the unpolluted soil. This was due to the fact that increased SEO pollution increases SEO constituents in the soil which makes the soil toxic. This was in line with the reports of Akpoveta *et al*. (2011) who stated that pollution becomes harmful when present in large quantities.

All compost amendments increased soil TP, protease, and Total Bacteria Count (TBC). Babalola (2019) reported that soil amendment with compost stimulate soil microorganisms to produce higher levels of cellulases, amylase and protease. Compost rate of 20 t/ha reduced soil THC and Pb, levels. The compost effect increased as compost rate increased. The compost effect was due to the capacity of compost to improve soil structure and fertility. This was supported by Cole *et al*. (1995) who stated that compost is widely used as a soil amendment to improve soil structure, provide plant nutrients and facilitate the re-vegetation of disturbed soil.

All microbial treatments increased soil TP, protease, TBC; and reduced the levels of THC and Pb, in soil. *Pseudomonas alcaligenes* and *B. thuringiensis* were more effective. These could be as a result of compost applied which supports the activities of microbes to increase the production of enzymes. This is in line with the United States Composting Council (2008), who reported that compost has the ability to bind heavy metals and thus reducing their absorption by plants; and that compost contains enzymes that can degrade some toxic organic compounds.

Total Hydrocarbon (THC) and Pb levels were increased in tomato roots with increase in pollution rates. This was so be-

cause as the pollution increased, the pollutants locked up essential nutrients necessary for plant growth; they become harmful and cause defective growth in plants. The significant increase witnessed in THC and Pb was attributed to the presence of organic pollutants and heavy metals in SEOs (Wang *et al*., 2000).

Application of 10 or 20 t/ha compost reduced root concentrations of THC and Pb. This was the effect of compost coming into play as compost has the potential to remediate polluted soils. Gestel *et al*. (2003) and Dutra *et al*. (2013) reported that total petroleum hydrocarbon contaminated soils were remediated by mixing the soil with matured compost of diverse genus. This could be due to the capacity of compost to improve the physical, chemical and biological properties of the soil as well as its capacity to bind and breakdown petroleum hydrocarbons which resulted in proper growth of tomato.

It was observed that *P. entomophila, P. alcaligene* and *B. thuringiensis* reduced root THC and Pb. This is the outcome of the activities of the microbes. It was supported by Gliessman (2006) who said that activities of soil microbes degrade pollutants, release nutrients into the soil and made them available for plant use.

Pollution increased THC and Pb, in tomato shoots. This was due to the presence of organic pollutants in the polluted soils that locked up essential nutrients necessary for tomato growth and development. Adu *et al*. (2015) noted that petroleum hydrocarbons alter the fertility status of soils and hence reduce their ability to support proper crop growth and development. The significant increase in THC, Pb were attributed to the presence of organic pollutants and heavy metals in SEOs (Wang *et al*., 2000).

Compost rate of 10 and 20 t/ha applied reduced the concentrations of shoot THC and Pb. This was due to the fact that compost has the capacity to improve soil fertility, bind heavy metals and breakdown hydrocarbons in the presence of microbes (USCC, 2008; Babalola *et al*., 2012)

Microbial inoculation of *P. entomophila, P. alcaligenes* and *B. thuringiensis* significantly reduced the concentrations of shoot THC and heavy metal (Pb) when compared to control (no microbe). However, *P. alcaligenes* and *B. thuringiensis* proved to be the most effective. This result justified the fact that the outcome of microbial activity on SEO polluted soils led to pollutants reduction and hence, proper crop development. This was in agreement with the report of Bonaventura and Johnson (1997) that microorganisms, as well as higher organisms can play an important role in the bioremediation of the concentration of metals, so that they become less available and less hazardous.

Pollution with 200 ml SEO increased fruit weight, while pollution rates of 200 and 400 ml significantly increased THC and Pb. The increase in fruit yield could be as a result of the high content of carbon and organic matter in the soil translocated to plant tissues. The significant increase witnessed in THC and Pb were attributed to the presence of organic pollutants and heavy metals in SEOs (Vwioko *et al*., 2006; Wang *et al*., 2000).

Compost applied at 10 or 20 t/ha increased weight of tomato fruits, and reduced THC. This could be due to the capability of compost to improve the physical, chemical and biological properties of the soil as well as its potential to bind and degrade pollutants which lead to higher yield and quality of tomato. According to Babalola (2019), compost improve yield of tomato, this is a consequence of increased availability of nutrients in compost.

Inoculation with B. thuringiensis reduced THC. This was in line with the reports of several researchers that microbes have the ability to unlock essential nutrients in the soil for plant uptake by the release of enzymes as well as the breakdown of hydrocarbons (Gliessman 2006; Adebusoye *et al*., 2007).

The interaction of compost application (10 and 20 t/ha) with all the microbial levels in 200 and 400 ml pollution rates significantly reduced THC and Pb concentrations in soil, root and shoot. It also increased fruit weight. The THC and Pb values reduced significantly with increase in compost rates. This was as a result of the fact that compost has the potential to enhance soil fertility in polluted soils by supporting the degradation of organic pollutants (USCC, 2008; Babalola *et al*., 2012).

Enrichment Coefficient (EC), Bio-concentration Factor (BCF) and Translocation Factor (TF) values of tomato plant grown in soil amended with compost and microorganisms in SEO polluted soil is less than one. This implies ineffective metal transfer suggesting that the plants accumulate metals in the roots and rhizomes more than in shoots or the leaves (Yoon *et al*., 2006).

5.0 Conclusion and Recommendation

The molecular characterisation showed that there are eight (8) distinct oil degraders identified in the SEO polluted soil which are *P.guariconensis, P. aureginosa, P. putida, P. entomophila, B. thuringiensis, P. alcaligenes, P. monteilii* and *Staphylococcus aureus*.

The microbial biomass N, C, P and residual soil total hydrocarbon revealed that 3 out of the identified oil degraders were efficient. They are *P. alcaligenes, P. entomophila,* and *B. thuringiensis.*

Bio-stimulation of SEO polluted soil indicated that 20 t/ha of compost is suitable for remediation. However, 10 t/ha also considerably remediated the polluted soil.

All the micro-organisms tested were able to bio-remediate the polluted soil. However, B. thuringiensis significantly remediated the polluted soil more than the other microbes.

Soil treatments with 10 t/ha + *B. thuringiensis*, 10 t/ha + *P. entomophila*, 10 t/ha + *P. alcaligenes*, 20 t/ha + *B. thuringiensis*, 20 t/ha + *P. entomophila* and 20 t/ha + *P. alcaligenes* reduced the level of THC and heavy metals (Pb) from SEO polluted soil; and in tomato vegetative parts significantly.

The results demonstrated that 10 t/ha of compost increased total Protein, protease and total bacteria count in soil by 243, 133 and 35% respectively; and reduced THC in root, shoot and fruit by 17, 32 and 25% respectively.

Soil treatments with 10 t/ha also reduced Pb in root, shoot and fruit by 28, 22 and 0% respectively.

Compost and microbe reduce THC and Pb levels in tomato fruits. However, THC in fruits was still above the FAO/ WHO tolerable limit (0.1 mg/kg) but Pb was below the tolerable limit (0.05 mg/kg).

The study revealed that tomato has phytoaccumulation capabilities as it accumulates Pb and hydrocarbons in the root than other parts in this order soil > root > shoot > fruit.

The interaction of bio-stimulation (Compost), bio-augmentation (Microbes) and phytoremediation (tomato plant) have proved to be effective in bio-remediating SEO polluted soil.

It is therefore recommended that farmers should apply compost rate of 10 or 20t/ha to SEO (other petroleum products) polluted farmland. Bioremediation of SEO polluted soils with any of these combinations: 10 t/ha + *B. thuringiensis*, 10 t/ha + *P. entomophila*, 20 t/ha + *B. thuringiensis* and 20 t/ha + *P. entomophila* is effective. The cultivation of tomato on SEO polluted soil along with bio-augmentation (Microbes)

and bio-stimulation (Compost) is feasible.

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