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Phytotoxicity and Microbial Activities of Crude-Oil Polluted Pristine Soil during Bioremediation with Saw Dust Amendment from Awka, Nigeria

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ABSTRACT

The continuous drive to increase the production, distribution and utilization of petroleum and natural gas products to meet the energy demands of the population is usually associated with some environmental pollution problems especially on the lithosphere (soil) and the physicochemical remediation approaches such as evaporation, dissolution, dispersion with chemical dispersants/emulsifiers, and photo-oxidation all found to be eco-harzardous and non-cost ineffective. Microbiological and Physico-chemical properties of pristine soil contaminated with crude oil were assessed using standard procedures. The influence of saw dust in the remediation of the polluted soil was evaluated. Phytotoxicity of the polluted soil was assessed using maize and beans seed germination test. Most frequently occurring microbial isolates obtained were identified using molecular typing. Bacteria isolated from the pristine soil were: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Klebsiella pneumoniae*. Fungi isolated were *Penicillium citrinnum*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Rhizopus stolonifer*. The most frequently occurring novel isolates were: *S. maltophilia, P. sordelli, B. thuringensis, F. solani, C. deightonni, P. citrinnum*, and *C. bertholletiae.* Percentage organic carbon content of the crude oil polluted soil was 21.50% while the nitrogen content was 0.90% with a pH value of 4.69. The Percentage organic carbon of the pristine soil was 0.750%, percentage nitrogen content was 2.71% with a pH value of 5.69. This research showed that phytotoxicity test carried out on the crude oil polluted soil distorted its physico-chemical parameters which has consequent toxicity on the soil, thus possibly hampering its fitness for use in crop production. Phytotoxicity test on the saw dust amended soil significantly supported plant growth with a high germination index (%1G) for both crops (maize and beans) therefore making the soil fit for use in crop production.

KEYWORDS: *Phytotoxicity, saw dust, bioremediation and microbial activities*

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INTRODUCTION

The continuous drive to increase the production, distribution and utilization of petroleum and natural gas products to meet the energy demands of the population is usually associated with some environmental pollution problems especially on the lithosphere (soil) and the physicochemical remediation approaches such as evaporation, dissolution, dispersion with chemical dispersants/emulsifiers, and photo-oxidation all found to be eco-harzardous and cost ineffective (Nwankwegu *et al.,* 2016a; Okafor and Nwankwegu, 2016).

In Nigeria, over 20 percent of crude oil spills result from vandalization of pipelines while transportation accounts for over 10 percent pollution in both marine and terrestrial ecosystems (Orji *et al*., 2012;

Nwankwegu *et al*., 2016a). Automobile spent engine oil and exhaust such as carbon (IV) oxide and carbon (II) oxide are other ways of environmental pollution. Pollution of the soil by these hydrocarbon components such as crude oil, diesel, kerosene, asphaltenes and the recalcitrant poly nuclear aromatic hydrocarbon (PAHs) has dramatically reduced agricultural productivity and yields through permanent soil sterility, leaching and erosion (Anozie and Onwurah (2001). Worldwide pollution, however, has been addressed by promising and emerging technology called bioremediation. The use of saw dust as amendment option to remediate crude oil polluted pristine soil in Awka, Nigeria, for a period of eight months was conducted in this research. This study thus assessed phytotoxicity, microbial and physico-chemical status of crude oil polluted pristine soil sample obtained from Awka, Anambra State, Nigeria, before and after bioremediation process using saw dust as a source of bioaugumentation and biostimulation.

Methods

Study Area:

Agricultural farmland located at Nnamdi Azikiwe University Awka, Anambra State Nigeria, was used for the study. Nnamdi Azikiwe University is located at 6.2437° N, 7.1219°E, Nigeria. Awka town, is situated at Anambra State, Nigeria; and is known for having agricultural-friendly soil which support growth of crops like cassava, corn, rice and yam.

Sample Collection

The soil sample 1000g was collected from the top surface soil (0-15cm) at Nnamdi Azikiwe University agricultural farmland at Ifite-Awka into a plastic pails already sterilized with 70% ethanol, and used for the entire study using modified method described by Eziuzor and Okpokwasili. (2009). The soil was sieved with Imm sieve and was polluted with 500ml of crude oil. The polluted soil was divided in two microcosms.

Determination of Pristine Soil Physico-chemical Parameters

Baseline physicochemical characteristics of the polluted soil were determined according to the combined methods of APHA (1998), Bento *et al*. (2005) and Nwankwegu *et al*. (2016a). The parameters determined included pH, moisture content, Organic carbon, total nitrogen, particle size distribution (i.e. percentage sand, silt, and clay), soil texture as well as heavy metals.

Phytotoxicity Assay of the Pristine Soil and crude oil polluted soil

Evaluation of agricultural performance of the two soils using seed germination as monitoring tool to measure phytotoxic level of the amended soil relative to the control was carried out. The method of seed germination and growth suggested by Millioli *et al*. (2009) with little modification in temperature and time was employed using two different agricultural crops; cereal grain of *Zea Mays* species and beans seed *Vicia faba* species (Fabaceae). 10g each of the two different experimental soil were placed in plastic-dishes. Ten seeds each of *Zea Mays* and *Vicia faba* respectively were distributed in the different dishes equally spaced. The plates were incubated for 4-5 days at room temperature. The soils containing the planted seeds were periodically moistened to check diffusional limitations of substrate supply and adverse physiological effect associated with cell dehydration as water penetrates the soil matrix and also to facilitate the swelling of the endosperm as well as the cotyledons and quicken germination. After this time, the number of germinated seeds was counted and the elongations of the roots were measured from the transition point among the hypocolite to its extremity, root elongations and shoot lengths were also measured.

The germination index (%IG) and percentage seed germination as suggested by Millioli *et al*. (2009) were calculated as thus:

$$\% IG = \frac{(\% SG) \times (\% GR)}{100} \quad (1)$$

$$\% SG = \frac{(\% EG)}{(\% CG)} \times \frac{100}{1} \quad (2)$$

$$\% GR = \frac{GERm}{GERCm} \times \frac{100}{1} \quad (3)$$

Where

% SG = Seed germination.

% GR = Growth of the roots.

% EG = Germination in saw dust amended soil.

% CG = Germination in control (pristine soil).

GERm = Elongation of roots in saw dust amended soil.

GERCm = Elongation of roots in control (pristine soil).

Microbiological Examination of Soil Samples

Isolation of Bacteria

Method described by Nwankwegu and Onwosi (2017) was used. Nutrient agar medium was used for the isolation of bacteria from the pristine soil. A 1g portion of the soil sample was diluted ten-fold in sterile water. 1 ml of 10^{-2} dilution factor was pour plated on nutrient agar medium. The plates were incubated for 24 h at room temperature. Afterwards, colonies that developed on the plates were counted and sub-cultured on fresh nutrient agar plates to obtain pure cultures. Pure cultures were stored on Nutrient Agar slants.

Biochemical Characterization of Bacteria Isolates

Several biochemical tests were carried out to characterize the bacterial isolates which included Gram stain, motility, indole, methyl red, voges Proskauer, citrate, catalase, coagulase, oxidase and sugar fermentation tests according to the methods described by Cheesbrough (2006) and Oliveira *et al*. (2006).

Isolation and Characterization of Fungi

The modified methods described by Anozie and Onwurah (2001) were used. A 0.1ml aliquots of the 10^{-2}dilutions of the soil sample were spread on triplicates of sterile Potato Dextrose Agar (PDA) plates using sterile glass spreader. The fungal media was amended with 0.5 mg/ml of Chloramphenicol to inhibit bacterial growth. The plates were incubated for 48 hours at room temperature (28±2°C). Colonies formed were counted and expressed in colony forming unit per gram CFU/g using the formular =

$$\frac{\text{No of colonies x amount used}}{\text{Dilution factor}}$$

Values were expressed in colonies forming unit per gram CFU/g

Preliminary fungal characterization were done by studying the cultural characteristics and employing the slide culture wet mount technique for evaluating the fungal microscopic features with reference to the Manual of Fungal Atlases according to Frey *et al*. (1979).

Molecular Characterization of Isolates

Molecular characterization was done using the protocols provided by Macrogen (2014). The identities of most occurring bacterial and fungal isolates were confirmed at Macrogen Inc., 10F, 254 Beotkkot-ro, Geumcheon-gu, Seoul, Republic of Korea, using the 16s rDNA and ITS rDNA Sequence Analyses for bacteria and fungi respectively.

Results

Microbial Examination of Crude Oil Contaminated Soil

Bacteria and Fungi isolated from the contaminated soil after addition of saw dust amendment were shown in Tables 1and 2 respectively. Bacteria isolated were: *Alcaligenes faecalis, Stenotrophomas maltophilia, Paeniclostridium sordelli, Corynebacterium amycolatum, Bacillus subtilis, Bacillus cereus, Bacillus thuringensis, Pseudomonas fluorescens, Flavobacterium indologenes, Streptococcus faecalis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Fungi isolated were: *Aspergillus flavus, Fusarium solani, Penicillium citrinnium, Geomyces pannorum, Acremonium fusidiodes, Aspergillus fumigatus, Cladosporium sphaerosporum, Curvularia heteropogonis, Penicillium griseofulvum, Rhizopus stolonifer, Candida utilis* and *Cochlobolus lunatus*. Bacteria and Fungi isolated from unpolluted pristine soil respectively. Bacterial isolated were: *Bacillus subtilis, Pseudomonas fluorescens, Alcaligenes faecalis, Klebsiella pneumoniae, Bacillus cereus, Bacillus thuringiensis* and *Pseudomonas aeruginosa.* Fungi isolated were: *R* Fungi isolated were *hizopus stolonifer, Aspergillus fumigates* and *Aspergillus flavus.*

Physico-chemical Examination of pristine and Crude Oil Contaminated Soil

Physico-chemical parameters of pristine and crude oil contaminated soil are shown in Table 3.

Table 1: Cultural and Biochemical Properties of Bacteria Isolated from Crude Oil Contaminated Soil

S/N	Cultural characteristics	Gram stain.	Citrate	Urease	Indole	Gelatin	Casein	MethylRed	Voges Proskuer	Catalase	Oxidase	motility	spot	Glucose	Sucrose	Lactose	maltose	Mannose	Xylose	Suspected Organisms
1	Whitish colonies with irregular edge	Gram negative rods	+	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	-	*Alcaligenes faecalis*
2	Slight yellow colonies on nutrie	Short Gram neg	+	-	-	+	+	-	-	+	-	+	-	+	+	+	+	-	+	*Stenotrophomas maltophilia*

	nt agar	ative rods																	
3	Cream small colonies 1-4mm which spread on the plate	Gram positive rods	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	*Paeniclostridium sordelli*
4	Creamy club shaped bacilli	Gram positive rods	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	*Corynebacterium amycolatum*
5	Large grey white colonies on nutrient agar	Gram positive rods in chain	+	-	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+	*Bacillus subtilis*
6	Large grey white colonies on nutrient.	Gram positive rods	+	-	-	-	+	-	+	+	-	+	+	+	+	-	+	-	-	*Bacillus cereus*
7	Large grey white colonies on nutrient agar	Gram positive rods	+	-	+	+	+	-	+	+	-	+	+	+		-	+	-	-	*Bacillus thuringiensis*
8	Green colonies on nutrient agar	Gram negative rods	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	*Pseudomonas fluorescens*
9	Orange pigmented coloni	Gram negative	-	-	+	+	+	+	-	+	+	-	-	+	-	-	+	+	-	*Flavobacterium indologenes*

	es	straight rods with rounded edges																	
10	Cream coloured colonies on nutrient agar, produced Beta haemolysis on blood agar	Gram positive cocci in chains	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	*Streptococcus faecalis*
11	Cream colonies on nutrient agar	Gram negative rods	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	*Pseudomonas aerugi nosa*
12	Cream colonies on nutrient agar	Gram negative rods	-	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	*Escherichia coli*
13	Cream colonies with black center on *Salmonella-Shigella* agar	Gram negative rods	+	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	+	*Salmonella typhi*
14	Greyish white	Gram neg	+	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	*Klebsiella pneu*

	colonies on nutrient agar	ative rods																		*monia e*

Table 2: Fungal Isolates from Crude Oil Contaminated Soil

S/N	Cultural characteristics	Microscopy	Organisms Isolated
1	Rapid growing white colonies that turned green, the reverse plate is cream on potato dextrose agar (PDA).	Hyphae is septate, conidia is globose	*Aspergillus flavus*
2	White cottonly colonies with fluffy growth, reverse is pale yellow on PDA.	Macro conidia are slightly curved and broad.	*Fusarium solani*
3	White folded colonies on SDA while the reverse is yellow.	Conidiophores bearing brush like conidia and hyphae are septate.	*Penicillium citrinnum*
4	Yellow to brown colonies that are powdery in texture reverse is brown on PDA.	Conidia formed in short chains of two to four arthroconidia	*Geomyces pannorum*
5	White to pale grey powdered colonies on PDA	Hyphae are septate with solitary phialites	*Acremonium fusidiodes*
6	Blue-green to grey green colonies while the reverse is pale on PDA.	Conidiophores tinted greenish with conidial heads.	*Aspergillus fumigatus*
7	Brownish black colonies on the surface and reverse on PDA	Septate hyphae with branching blastoconidia	*Cladosporium sphaerosperum*
8	White to olive brown colonies on surface and reverse on PDA.	Hyphae are septate with brown conidiophores with a central dark cell.	*Curvularia heteropogonis*
9	Green colour on the surface with pale colours on the reverse on PDA.	Septate hyphae, phialide are in brush like clusters.	*Penicillium griseofulvum*
10	White colored colonies becoming grey-brown on the surface, reverse is pale on PDA	Hyphae are non-septate, Rhizoids and stolons are present.	*Rhizopus stolonifer.*
11	Cream rapid growing postly colonies on PDA.	Non –filamentous vegetative cells, aerial hyphae in groups of two to three short conidiophore. Gram-positive budded yeasts.	*Candida utilis*
12	Brown to black velvety colonies on Potato dextrose agar	Septate conidiophores in groups with swollen bases has three septa and four cells.	*Cochlobolus lunatis*

Table 3: Physico-Chemical Properties of Soil Samples

Parameters	Pristine Soil Unpolluted (Control)	Crude oil Polluted Soil	Saw Dust Amended Soil
% Nitrogen	2.71	0.90	4.1
Organic carbon %	0.750	21.50	5.5
% silt	7.52	7.52	7.52
% sand	65.22	65.22	65.22
% clay	27.26	27.26	27.26
pH	5.69	4.69	6.1
Ca cmol/kg	0.0667	0.0667	0.001
Mg cmol/kg	0.0778	0.0778	0.001
K cmol/kg	0.0274	0.0274	0.00
Na cmol/kg	0.02327	0.02327	0.00
Magnesium ppm	18.671	18.671	10.2

Potassium ppm	21.352	21.352	10.34
Sodium ppm	107.06	116.06	3.61
Calcium ppm	26.646	20.646	-
Phosphorus	6.630mg/kg	6.110mg/kg	21.2
Moisture content %	12.419	18.419	9.8
Soil texture classification	Sandy loam	Sandy loam	Sandy loam

Discussion

This research work sought to evaluate the impact of saw dust on phytotoxicity and microbial compositions and resultant Physico-Chemical properties of crude oil polluted soil in agricultural farmland from Awka, Nigeria. The most frequently occurring microorganisms isolated from the crude oil polluted soil as shown in Tables 1 and 2 were: *S. maltophilia, P. sordelli, B. thuringensis, F. solani, C. deightonni, P. citrinnum*, and *C. bertholletiae.* A good number of these frequently occurring organisms (*S. maltophilia, C. deightonni* and *C. bertholletiae*) has not been reported by authors who have published research findings on microbial communities found in crude oil polluted soil in Nigeria. Ogbonna *et al*. (2020) reported *Bacillus, Penicillium* and *Fusarium* as most frequently occurring isolates from crude oil polluted soil sample collected from Diobu area of Port-Harcourt, Nigeria. These microbes reported by Ogbonna *et al*. (2020) correspond with our research findings. Xu *et al*. (2018) reported novel bacterial strains such as *Alkanindiges, Alteromonas, Dietzia* and *Kocuria* which differed from the strains isolated in this work.

The physico-chemical analysis of crude oil polluted soil samples showed that the soil had acidic pH, high organic carbon content and relatively high carbon-nitrogen ratio. Crude oil polluted soil did not support plant growth due to the hypertoxic hydrocarbon contents of the crude oil. According to Devatha *et al*. (2019) significant decrease in pH range for crude oil contaminated soil comes about as a result of reaction of hydrocarbons in the crude oil with the soil salts and minerals.

After the bioremediation process, the physico-chemical parameters of the soil amended with saw dust had carbon-nitrogen ratio in the right proportion that could encourage favorable agricultural yield. However, phytotoxic test carried out showed that maize and beans seeds planted in the saw dust amended soil gave the best germination index (%IG) for both crops.

Conclusion

This research has been able to show the positive effect of saw dust amendment option on the amelioration of phytotoxicity and physico-chemical properties of agricultural soil from Awka, Nigeria, polluted with crude oil. The polluted soil significantly supported plants growth after the bioremediation exercise.

REFERENCES

[1] Agarry, S. E., Aremu, M. O. and Aworanti, O. A. (2013). Kinetic Modelling and Half Study on Enhanced Soil Bioremediation of Bonny light crude oil Amended with Crop and Animal-Derived Organic Wastes. *Journals of petroleum environmental biotechnology,* 4:137.

[2] Anozie, O. and Onwurah, I. N. E. (2001). Toxic Effects of Bonny Light Crude Oil in Rats after Ingestion of Contaminated Diet. *Nigerian Journal of Biotechnology and Molecular Biology (Proceedings supplement)*, 16:1035–1085.

[3] APHA, (1998). Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington DC, USA.

[4] Bento, F. M., Camargo, F. A. O., Okeke, B. C. and Frankenberger, W. T. (2005). Comparative Bioremediation of Soils Contaminated with Diesel Oil by Natural Attenuation, Biostimulation and Bioaugmentation. *Bioresource Technology*, 96: 1049–1055.

[5] Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries (Part 2, 2nd ed.). UK: Cambridge University Press. p. 62-313.

[6] Chorom, M., Sharifi, H. S. and Motamedi, H. (2010). Bioremediation of A Crude Oil-Polluted Soil by Application of Fertilizers. Iranian Journal of Environmental Health Science and Engineering, 7 (4): 319-326.

[7] Devatha, C. P., Vishal, A. V. and Rao, P. C. (2019). Investigation of Physical and Chemical Characteristics on Soil Due to Crude Oil Contamination and Its Remediation. *Applied Water Science*, 9:89.

[8] Eziuzor, C. S. and Okpokwasili, G. C. (2009). Bioremediation of Hydrocarbon Contaminated Mangrove Soil in a Bioreactor. *Nigerian Journal of Microbiology*, 23:1777 – 1791.

[9] Frey, D., Oldfield, R. J. and Bridges, R. C. (1979). A Colour Atlas of Pathogenic Fungi. Wolf medical publications Ltd London.

[10] Ijah, U. J. J. and Antai, S. P. (2003). Removal of Nigerian Light Crude Oil in Soil Over A 12 – Month Period. *International Biodeterioration and Biodegradation* 51: 93 – 99.

[11] Millioli, V. S., Servulo, E. L. C., Sorbal, L. G. S., De Carvalho, A. D. (2009). Bioremediation of crude oil bearing soil: evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil biodegradative efficiency. *Global NEST Journal*. 11(2): 181 – 188.

[12] Nwankwegu, A. S. and Onwosi, C. O. (2017). Bioremediation of Gasoline Contaminated Agricultural Soil by Bioaugmentation. *Environmental Technology and Innovation*, 7:1-11.

[13] Nwankwegu, A. S., Onwosi, C. O., Orji, M. U., Anaukwu, C. G., Okafor, U. C., Azi, F. and Martins, P. E. (2016a). Reclamation of DPK Hydrocarbon Polluted Agricultural Soil Using A Selected Bulking Agent. *Journal of Environmental Management*, 172: 136 – 142.

[14] Nwankwegu, A. S., Orji, M. U., Onwosi, C. O. (2016b). Studies on Organic and In-Organic Biostimulants in Bioremediation of Diesel-Contaminated Arable Soil. *Chemosphere*. 162: 148 – 156.

[15] Ogbonna, D. N., Douglas, S. I. and Awari, V. G. (2020). Characterization of Hydrocarbon Utilizing Bacteria and Fungi Associated with Crude Oil Contaminated Soil. *Microbiology Research Journal International*, 30(5): 54-69.

[16] Oliveira, G., Ribeiro, E. and Baroni, F. (2006). An Evaluation of Manual and Mechanical Methods to Identify *Candida* spp. from Human and Animal Sources. *Revised Institute of Medicine and tropics Sao- Paulo* 48(6):311-315.

[17] Orji, F. A., Ibiene, A. A. and Dike, E. N. (2012). Laboratory Scale Bioremediation of Petroleum Hydrocarbon-Polluted Mangrove Swamps in the Niger Delta Using Cow Dung. *Malaysian Journal of Microbiology*, 8:219 – 228.

[18] Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H. and Yu, H. (2018). Petroleum Hudrocarbon Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Frontiers in Microbiology*, 9: 2885.