International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 6, September-October 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Microbiological and Physico-chemical Examination of Crude Oil Contaminated Soil from Awka Anambra State Nigeria

Anagboso, M. O.; Orji, M. U.; Ikele, M. O.

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria

ABSTRACT

Crude oil is a complex mixture of aliphatic and aromatic hydrocarbons that causes a variety of risks when released into agricultural and aquatic environment. This oil can alter population dynamics and disrupt tropic integrations and the structure of natural communities within ecosystems during spills. Microbiological and Physico-chemical properties of crude oil and pristine soil samples were assessed using standard procedures. Most frequently occurring microbial isolates obtained were identified using molecular typing. Bacteria isolated from the contaminated 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 contaminated soil was 21.50% while the nitrogen content was 0.90% with a pH value of 4.69. This research showed that crude oil contamination of agricultural soil distorts its physico-chemical parameters which has consequent toxicity on the soil, thus possibly hampering its fitness for use in crop production.

KEYWORDS: *Bioremediation, Crude oil and soil examination*

How to cite this paper: Anagboso, M. O. | Orji, M. U. | Ikele, M. O. "Microbiological and Physico-chemical Examination of Crude Oil Contaminated Soil from Awka Anambra State Nigeria" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-6, October 2021, pp.635-642, URL: www.ijtsrd.com/papers/ijtsrd46442.pdf

IJTSRD46442

INTRODUCTION

Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons that causes a variety of risks when released into agricultural and aquatic environment. It is physically, chemically and biologically harmful to living organisms in the soil because of the presence of many toxic compounds, such as polycyclic aromatic hydrocarbons, benzene and its substituted cycloalkane rings, in relatively high concentrations. This oil can cause chronic sub-acute toxicological effects (reduced growth and reproduction, poor health, low recruitment rates), which can alter population dynamics and disrupt tropic integrations and the structure of natural communities within ecosystems (Agarry *et al.,* 2013). The fate and effects of spilled crude oil and its products in soils have already been the subject of several studies (Ijah and Antai, 2003; Nwankwegu *et al*., 2016b).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) which consequently affects its physico-chemical properties and microbial population diversity.

In Nigeria, over 20 percent of crude oil spills result from vandalism 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). Awka town, is situated at Anambra State, Nigeria; and is known for having agricultural-friendly soil which support growth of

crops like cassava, corn and rice. This study thus assessed the microbial and physico-chemical status of crude oil contamination of soil obtained from Awka, Anambra State, Nigeria.

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.

Sample Collection

The soil sample was collected from the top surface soil (0-15cm) of Nnamdi Azikiwe University agricultural farmland at Ifite-Awka into a plastic pail 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 I mm sieve and was used at 1:1 ratio for the preparation of the composite samples.

The Bonny light crude oil was obtained from Nigeria National Petroleum Corporation, Port Harcourt, Nigeria and 500 ml of the crude oil was used to contaminate 1000 g of soil sample.

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 crude oil polluted 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 =

No of colonies x amount used
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.

Determination of Pristine and Contaminated Soil Physico-chemical Parameters

Baseline physicochemical characteristics of the polluted soil were determined according to the modified methods of APHA (1998), Bento *et al*. (2005) and Nwankwegu *et al*. (2016a). These 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.

Results

Microbial Examination of Crude Oil Contaminated Soil

Bacteria and Fungi isolated from the contaminated soil were also shown in Tables 1and2 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*.

Physico-chemical Examination of Crude Oil Contaminated Soil

Physico-chemical parameters of the 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	VogesProuskuer	Catalase	Oxidase	motility	spot	Glucose	Sucrose	Lactose	maltose	Manose	Xylose	Suspected Organisms
1	Whitish colonies with irregular edge	Gram negative rods	+	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	-	*Alcaligenes faecalis*
2	Slight yellow colonies on nutrient agar	Short Gram negative rods	+	-	-	+	+	-	-	+	-	+	-	+	+	+	+	-	+	*Stenotrophomas maltophilia*
3	Cream small colonies 1-4mm which spread on the plate	Gram positive rods	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-	*Paenicl ostridium sordelli*
4	Creamy club shaped bacilli	Gram positive rods	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	*Coryneb acterium amycolatum*
5	Large grey white colonies on nutrient agar	Gram positive rods in chain	+	-	-	+	+	-	+	+	-	+	+	+	+	-	+	+	+	*Bacillus subtilis*
6	Large grey	Gram	+	-	-	-	+	-	+	+	-	+	+	+	+	-	+	-	-	*Bacillus*

	white colonies on nutrient.	positive rods																		*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 colonies	Gram negative straight rods with rounded edges	-	-	+	+	+	+	-	+	+	-	-	+	-	-	+	+	-	*Flavobacterium indologenes*
10	Cream coloured colonies on nutrient agar, produced Beta haemolysis	Gram positive cocci in chains	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	*Streptococcus faecalis*

	on blood agar																		
11	Cream colonies on nutrient agar	Gram negative rods	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*
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 colonies on nutrient agar	Gram negative rods	+	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	*Klebsiella pneumoniae*

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 Parameters of Crude Oil contaminated soil

Parameters	Crude oil Polluted Soil
% Nitrogen	0.90
Organic carbon %	21.50
% silt	7.52
% sand	65.22
% clay	27.26
pH	4.69
Ca cmol/kg	0.0667
Mg cmol/kg	0.0778
K cmol/kg	0.0274
Na cmol/kg	0.02327
Magnesium ppm	18.671
Potassium ppm	21.352
Sodium ppm	116.06
Calcium ppm	20.646
Phosphorus	6.110mg/kg
Moisture content %	18.419
Soil texture classification	Sandy loam

Discussion

This research work sought to evaluate the microbial compositions and resultant physico-chemical impact of crude oil contamination of agricultural soil from Awka, Anambra, State. The most frequently occurring microorganisms isolated from the crude oil contaminated soil as shown in Tables 1 and 2 are *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 the soil samples showed that the crude oil contaminated soil had acidic pH, high organic carbon content and relatively high carbon-nitrogen ratio. 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. They went on to explain that increased organic carbon content in the contaminated soil is mainly caused by the total organic carbon concentration from the crude oil which is deposited into the contaminated soil leading to agronomical addition of the carbon content to the soil. This organic carbon will influence plant growth directly or indirectly based on nutrient availability; which in the other words, implies that increased organic carbon concentration in soil will be beneficial if more microorganisms are present in the soil to hydrolyze them, resulting in decreased total nitrogen and available phosphorous concentration, and consequently, should promote plant growth (Devatha *et al.*, 2019).

Conclusion

This research has been able to identify the impact of crude oil contamination on the physico-chemical properties of agricultural soil from Awka and the resultant microorganisms that inhabit the contaminated soil. It has also shown that these organisms are known hydrocarbon degraders with a few novel ones like *S. maltophilia, C. deightonni* and *C. bertholletiae.*

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