



Mycoflora of Crude Oil Polluted Soils of Ukwa West of Abia State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Bioremediation of soil play a critical role in removing pollutants from crude oil polluted soil. To investigate the mycoflora of crude oil polluted area of Ukwa West Local Government in the present Abia State.

Methodology: Portions of the soil samples collected from three (3) locations were plated out separately on Yeast Starch Agar, Starch Agar, and Cellulose Agar using soil plate methods. The culture plates were examined after 5-7 and 14 days for the presence of fungi. Pure cultures of the isolates were obtained by subculturing and the physico-chemical properties of the soil samples were determined using standard methods.

Results: Twenty species (20) of fungi were isolated from the crude oil polluted soil sample and represented by their respective genera. Eight (8) species of *Aspergillus* representing 40%, two (2) species of *Thermomyces* representing 10% each, and one (1) species of *Penicillium*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Trichoderma*, *Scopulariopsis*, *Sporotrichum* and *Basipetospora* representing 5% each. *Aspergillus* species were predominant which include among others *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. oryzae*, *A. terreus*. The physico-chemical properties of the soil sample were found to be varied and have affected the distribution and population of fungi. The pH values ranged from 4.81- 5.58 as compared to the control ranging from 5.72- 6.50 for soil samples A, B and C. The moisture content values ranged between 26.46-29.59% as compared to the control ranging from 29.41-32.51%. The soil was found to be high in organic

matter content with values of 70.3-82.7% as compared to the control which was 94% for each of the locations.

Conclusion: Crude oil polluted soils of Ukwa West of Abia State, Nigeria is rich in fungal biodiversity with the soil's samples having different physico-chemical properties.

Keywords: Fungi; polluted soil; crude oil; yeast starch agar; cellulose agar; *Aspergillus*.

1. INTRODUCTION

The economic benefits associated with crude oil exploration cannot be over emphasized. However, there have been established hazardous effects (both direct and indirect) of these explorations and exploitations of crude oil on human health [1]. The environmental impact of crude oil exploration exploitation is the main consequences of economic development and civilization in a third world country like Nigeria. These consequences include incidental spills on the environment which affects both the microbial community and ecosystem composition of the soils, which as well alters the biogeochemical cycles and in effect affects soil fertility and environmental quality [2, 3]. Wamedo et al. [2] also reported that the incidences of environmental pollution as a result of high rate of petroleum related activities and are associated with oils spills following rupture of pipelines, illegal bunkering, pipeline vandalization, oil well blow outs and tanker accidents.

Soil is composed of five major components: mineral matter, water, air, organic matter and living organisms. The quantity of these constituents is not the same in all soils but varies with locality [4]. The vast different in the composition of soils, physical characteristics and the agricultural practices by which they are cultivated, result in corresponding large differences in microbial population both in the numbers and kinds [5]. Soil is the reservoir of a variety of microorganisms ranging from bacteria to fungi, actinomycetes, algae, viruses and protozoa. These microorganisms play important roles in the balance of soil ecosystems, bioremediation, adjusting of energy flow, cycling of nutrients, organic matter transfer and also on the growth and development of agricultural crops. Soil microorganisms play a major role in the biodegradation of hydrocarbons thereby helping in the clean-up of oil spills in crude oil polluted areas [6, 7]. There have been reports in the use of microorganisms in the bioremediation of crude oil polluted sites [8, 9, 10, 11, 12].

Fungal species have been used very much in biodegradation of hydrocarbons following the

reports of Andrea et al. [11]. Fungal bioremediation is usually suggested due to its cost efficiency as compared to other remediation technologies. The use of fungal remediation is usually economical due to the fact that they easily grow on agricultural and industrial wastes such as sawdust, rice straws, millet and guinea corn straws and corn cobs as well as the ease with which they are utilized in the clean-up exercise [13]. Recently, there has been increase in the use of indigenous fungi and other microorganisms for the clean-up of crude oil contaminated sites. This research work therefore was designed to use the indigenous fungal isolates from crude oil polluted sites in Ukwa West Local Government Area of Abia State, Nigeria to attempt to clean-up the oil spills using the isolates with highest efficiencies in enzyme biodegradation.

2. MATERIALS AND METHODS

2.1 Sample Collection and Fungal Isolations from Soil Samples

The areas selected for the study included Ukwa West Local Government Area in Abia State, Nigeria. Its headquarters is the town of Dike Ikpe. It has an area of 271 km² and a population of 88, 555 as at the 2006 census. The Local Government is the only crude oil producing area in Abia State. Its oil producing communities include Owaza, Uzuaku, Umuokwor, Umuahala and Umuorie. The Local Government Area is bounded in the North by Osisioma Ngwa, at North-east by Ugwuagbo, at the East by Ukwa East, at the West by Rivers State and at the South by Imo State

Soil samples collected randomly from each of the three locations in each site were mixed together in order to form a composite sample. The soil samples were plated out for the assessment of their fungal contents using soil dilution plate methods of Warcup [14]. Each soil sample was plated out on Starch Agar (SA), Yeast Starch Agar (YSA), Eggins & Pugh Cellulose Agar (E&PA) and Czapek-Dox Agar at different ranges of temperature 25°C, 37°C, & 45°C. Soil samples

collected from non-polluted sites served as control. The plates were examined for fungi after 4 days up to 14 days of incubation. The rough cultures were sub-cultured severally in order to obtain pure culturing and the culture plates were examined under the microscope to determine the number of fungi colonies. Scorings were made for each fungus colony that appeared in the plate and the result was subjected to statistical analyses. The experiments were replicated five times.

2.2 Identification of the Fungal Isolates

Each isolate was identified based on the microscopic features of its sporulating structures. Other characteristics observed included shape, color, size of the fungal hyphae, spores and fungal colonies, elevation and outline of the fungal colonies. Others include the nature of the hyphae such as being septate or non-septate and as well as the fruiting bodies [15]

2.3 Determination of the Physico-Chemical Properties of the Soil Samples

2.3.1 pH determination

The method of Ogbonna and Pugh [15] was used for the pH determination. A weight of 30 grams of each soil sample was weighed out. The soil samples were separately mixed with 100ml sterile water and well shaken in order to obtain soil suspension. The pH meter was first standardized using pH 4 and 7 buffer solutions before using. The pH value for each of the soil suspension was read directly from the scale. The average pH values of 3 replicates of each soil were determined and recorded.

2.3.2 Determination of percentage moisture content

For the moisture content determination, a weight of 30 grams of soil from each soil sample was dried to a constant weight in hot air oven set at 110 °C. The percentage moisture contents of the soil samples were determined in triplicates and then recorded [15].

2.3.3 Determination of percentage organic matter content

The organic matter content determination followed the methods of Ogbonna and Pugh [15]. Soil samples (30 grams) previously dried to a constant weight in hot air oven set at 110°C were used in the determination of the percentage (%)

organic matter content. The soil samples were put in porcelain crucibles and the crucibles were placed in a muffle furnace and heated at 400°C for 3 hours. The samples were cooled and the percentage organic content of the soil samples was determined in triplicates and then recorded.

2.3.4 Determination of percentage soil water holding capacity

The method of Ogbonna and Pugh [15] was also used for the assessment. Each of the soil samples were saturated with water in separate glass funnels. Each of the glass funnel was covered with perforated filter paper to prevent evaporation and to maintain atmospheric pressures on the soil and allowed for free drainage for 48 hours. The percentage water holding capacity of the soil samples was determined in triplicates as the differences in the weight of water at soil water holding capacity and the values were recorded.

3. RESULTS AND DISCUSSION

3.1 Fungal Isolation

A total number of 38 isolations were obtained from the analysis of the 30 soil samples. The identification of these isolates resulted into 20 species belonging to 12 genera of (Table 1). Among the identified species, 8 belonged to *Aspergillus*, 2 to *Thermomyces* and 1 species each to the genus of *Basipetospora*, *Cladosporium*, *Cunninghamella*, *Curvularia*, *Mucor*, *Pencillium*, *Scopulariopsis*, *Sporotrichum*, *Trichoderma* and *Trichophyton*. *Aspergillus* species were found to be more abundant contributing to 40% of the isolates, followed by *Thermomyces* having 20% of the isolates. The other species has 5% each of the isolates. Location C had the highest number of isolates (16), followed by location A (15). Location B had the least number of isolates (7). The details of the isolation experimental results are presented in Table 1. The details of some of the fungal isolates are presented in Plates 1a-1d. Many reports have it that all fungi recorded from this study were known as inhabitants of different soils [16,17,18,19]. However, these authors also recorded *Aspergillus* sp as the most encountered fungal species from their studies, similar to our findings.

3.2 Physicochemical Parameters of the Experimental Soil Samples

The results of the ecological parameters of the soils samples including pH values, percentage

moisture and organic matter contents, Percentage water Holding capacity and soils color are shown in Table 2. It was observed that location A had very acidic soil with a lower pH value of 4.81. Location B and C recorded higher pH values of 5.58 and 5-15 respectively. These results are indicating that the soils were acidic.

The soil samples from location C (Fig. 3) had the lowest percentage moisture content of 1.23 %, followed by location B and C with the values of 4.21 % and 4.61 % respectively. In the case of organic matter content, location A had the highest with 8.99 % while location B had lowest value of 7.34 %. The soils from location C had the highest percentage water holding capacity of 17.27 % while the least value of percentage water holding capacity obtained was for location B with 15.02 %. The soils from the 3 locations were found to be dark brown in colour. However, differences in soil pH, moisture content and organic matter content values of the different sampling locations were observed to be statistically significant [14]. The pH of the soil samples was at ranges that support fungi growth in culture. Fungi as a group tolerate a wide pH range, but some fungi are more tolerant to acidic soils. As compared to bacteria they can tolerate a wide range of pH 4-8 [15] which also correlates with the findings in this study (Fig. 1). As

indicated in this study, location A which the soil was highly acidic (pH 4.81) had high fungal plate count of 15. The highest plate count of 16 was recorded for location C probably because of the pH which was still within acidic range and high organic matter content of the location. The slightly acidic (pH 5.58) condition of location B (Fig. 2) could possibly be responsible for the fewer number of isolates of 7 from the location. Fungi are known to dominate acid soils but can also tolerate pH of beyond 9.0 [20]. The high moisture content of the soil samples must have contributed to the diversity of fungi isolated from the locations. Donnely et al. [20] reported soil moisture to be more important for microorganisms' growth than temperature and pH.

The organic matter contents of the soil which is the main source of utilizable substrates for the fungi plays a significant role as high number of the fungal species were recovered from locations A and C. Humus (organic matter) rich soils have large fungal population than soil poor in humus [21,22]. This also correlates with finding of this study as shown in Figs 1, 2 and 3. Singh and Sharma [23] reported the usefulness of microorganisms such as Actinomycetes, bacteria and fungi in the decomposition of organic matter as a result of their enzymatic activities which

Table 1. Mycoflora from three different locations of crude oil polluted sites

S/N	Fungal isolates	Sites			Total
		A	B	C	
1	<i>A. candidus</i>	-	-	+	1
2	<i>A. clavatus</i>	+	-	+	2
3	<i>A. flavus link</i>	-	-	+	1
4	<i>A. fumigatus</i>	+	+	+	3
5	<i>A. niger sp</i>	+	+	+	3
6	<i>A. oryzae</i>	-	-	+	1
7	<i>A. parasiticus</i>	+	-	-	1
8	<i>A. terreus</i>	+	+	+	3
9	<i>Basipetospora sp</i>	+	+	+	3
10	<i>Cladosporium sp</i>	+	-	+	2
11	<i>Cunninghamella sp</i>	+	+	+	3
12	<i>Curvularia sp</i>	+	-	+	2
13	<i>Mucor sp</i>	+	-	+	2
14	<i>Pencillium sp</i>	+	-	+	2
15	<i>Scopulariopsis sp</i>	+	-	+	2
16	<i>Sporotrichum sp</i>	+	-	-	1
17	<i>Trichoderma sp</i>	+	+	-	2
18	<i>Thermomyces ibadanensis</i>	-	-	+	1
19	<i>T. lanuginosus</i>	-	-	+	1
20	<i>Trichophyton rubrum</i>	+	+	-	2
Total		15	7	16	38

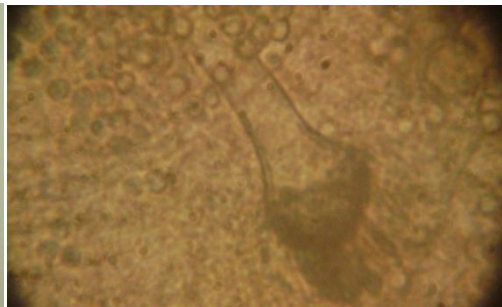
A, B, C = site locations from where the soil samples were collected + = Present, - = Absent

favors the elimination of organic wastes and then provide beneficial metabolic products to the soil. Microorganisms such as fungi have been used in the successful management of sustainable

agricultural resources to satisfy the human needs. They also help to enhance environmental quality and conserving natural resources [24].



Plate 1a. Culture of *Aspergillus candidus*



A. candidus



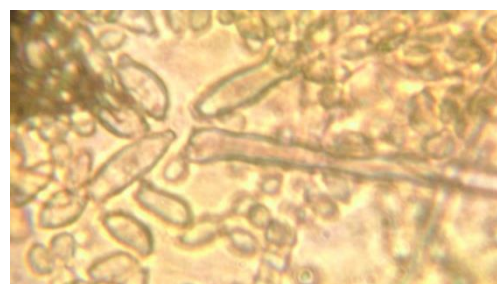
Plate 1b. Culture of *Aspergillus fumigatus*



A. fumigatus



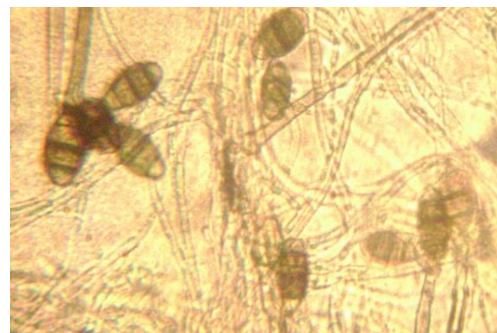
Plate 1c. Culture of *Cladosporium sp*



Cladosporium sp



Plate 1d. Culture of *Curvularia sp*



Curvularia sp

Table 2. Colonial and microscopic characteristic of some fungal isolates

Characteristics	<i>Aspergillus terreus</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>Curvularia sp</i>
Morphology of reproductive structure	Conidiophore terminated in columnar vesicle Biseriatephialidles arose from the vesicle conidia born on the phialides	Conidiophores bone from surface hypha long sometimes sinuous with colorless thin smooth wall enlarging gradually into phialides from vesicle to reverse is usually white	Conidiophores bone from surface hyphae long with heavy, hyaline smooth walls. Vesicles spherical, usually bearing closely packed metuleaphilades long born in large radiate heeds	Macroconidia are arranged in cluster at the tip of the conidiophore. They are curvularia with 3 transverse septa
Appearance PDA medium pigmentation	Sand brown colour mycelia is white reverse is uncoloured	Veutinous bluish colour colonies into head are diagnostic pyriforru	Spherical black conidial derived from colonies which show little or no colour	Bark brown and velvety, reverse, in near black
Macroconidia	None	None	Abundant macroconidia which tapper at both ends	
Hypha	Septate	Septate	Septate	Septate

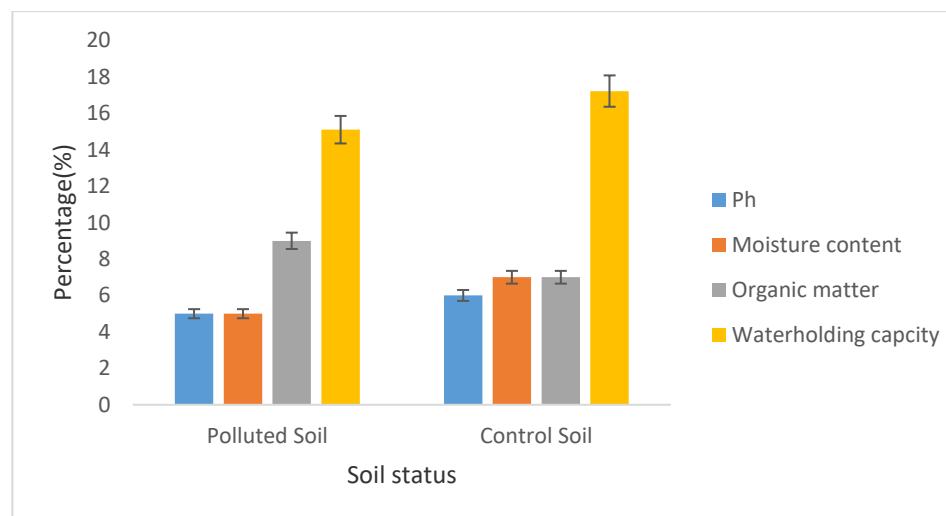


Fig. 1. Analytics of physico-chemical of crude-oil polluted soil samples in Location A

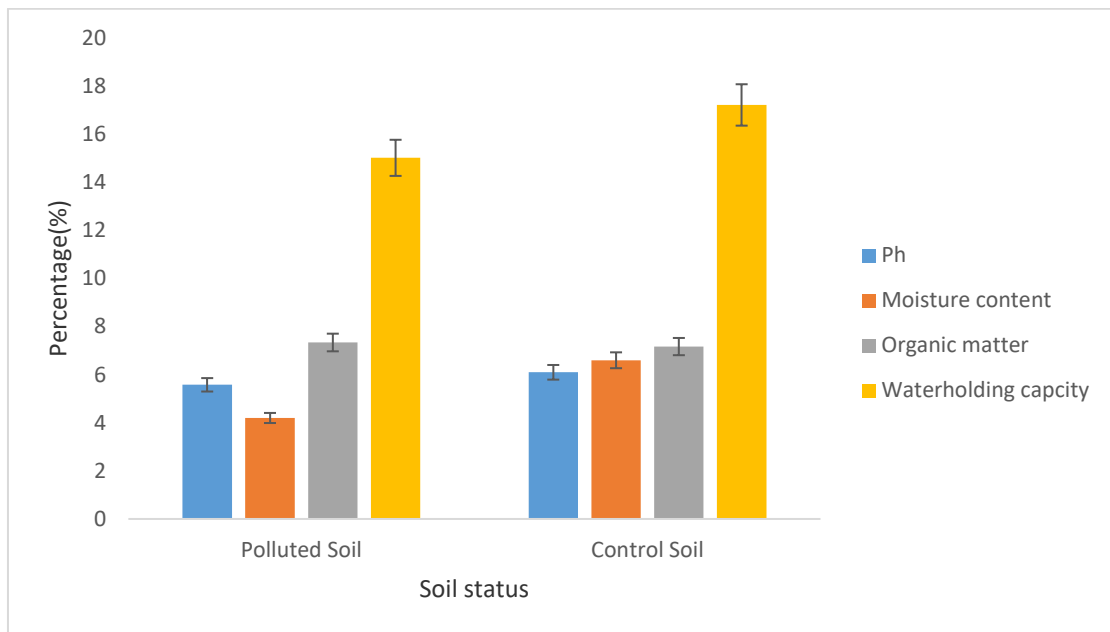


Fig. 2. Analytics of physico-chemical of crude-oil polluted soil samples in Location B

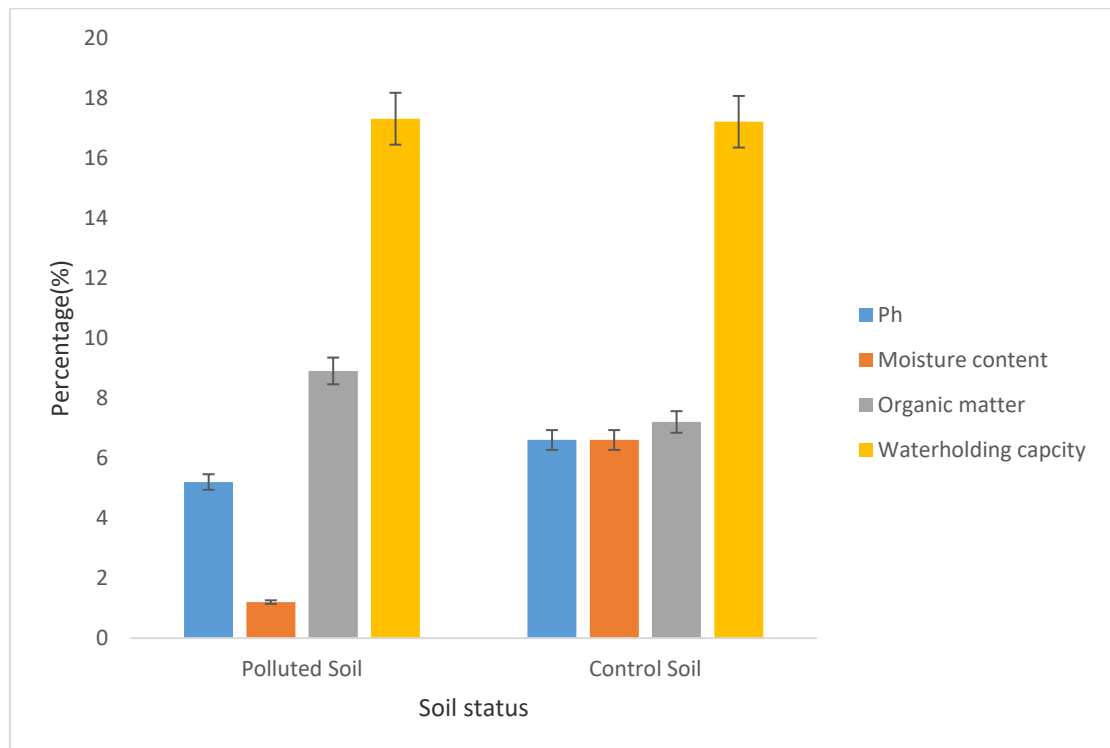


Fig. 3. Analytics of physico-chemical of crude-oil polluted soil samples in Location C

4. CONCLUSION

Crude oil polluted soils of Ukwu West of Abia State, Nigeria is rich in fungal biodiversity. These fungal isolates will help in building

indigenous Biological Directory of fungi in this part of the world as well as providing insights for bioremediation of crude oil spill areas in Nigeria using these strains.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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