Characterization of Indigenous Microorganisms Associated with Crude Oil-polluted Soils and Water Using Traditional Techniques

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ABSTRACT
A study was conducted to isolate and identify bacteria and fungi associated with crude oil-polluted sites using traditional techniques. Environmental samples were collected from Awoye, Mese and Oluwa villages in Ondo State and three different flow stations (Agbada-Aluu shell, Obite and Bonny) in Rivers State. Four plate technique was used for the analyses of microbial population. The bacterial isolates were identified by morphological and biochemical characterization using the taxonomy scheme of Bergey’s Manual of Determinative Bacteriology while identification of fungi was based on the microscopic and macroscopic features of the hyphal mass, nature of the fruiting bodies and the morphology of cells and spores. The bacterial load of the Obite water was highest (33.00±1.0 CFU mL⁻¹) and that of the Oluwa polluted water was lowest (10.00±0 CFU mL⁻¹). Soil samples collected from Mese, Oluwa and Awoye had fungal counts of 15±3, 4±2 and 16±3 SFU g⁻¹, respectively while the fungal population of the water samples varied from 8.67±2.1 SFU mL⁻¹ (Oluwa) to 15.00±1.0 SFU mL⁻¹ (Awoye). Seven bacteria each and fourteen fungi were obtained from Ondo and Rivers States. The advent of molecular biology in the 1980’s has no doubt provided new set of tools to identifying microorganisms to the specie as well as the strain of individual microorganisms. However, traditional techniques are useful especially in laboratories where there are no molecular biology facilities. Further research is necessary to assay for the degradative ability of the microorganisms.

Key words: Microorganisms, bioremediation, traditional techniques, molecular techniques, characterization

INTRODUCTION
Nigeria, the most populous and largest country in Africa, is located between latitude 4 and 14° north and longitude 2.45 and 14.30° east. The total water area is 15,000 sq km while that of land is 910,768 km² (CIA, 2009). The Nigerian coastal environment is made up of rich and diverse ecosystems, natural and large human resources. Its economy is largely dependent on the oil sector because most of the revenue generated in Nigeria comes from the proceeds of petroleum and its products. The oil sectors are majorly located in the Niger Delta which is situated in the south-eastern part of Nigeria. Subsistence farming and fishing are the mainstay of the people, out of which 75% rely on natural endowments for a living (Agbogidi et al., 2005). Statistics have shown that in the last 30 years, more than 400,000 tons of oil had spilled into the creeks and soils, thereby, devastating the environment of the fertile Niger Delta in Nigeria (CIA, 2009). The major causes of oil spills in the Niger Delta are due to anthropogenic activities and ageing facilities. Oil spills has detrimental effects on both the terrestrial and aquatic environment, particularly in the oil producing areas of the country. Nutrient immobilization following crude oil spill has been reported
by Siddiqui and Adams (2002). Oil pollution tends to cause drastic slow down in vegetation and recolonization of uncolonized habitats. The development of kidney and liver diseases, cancer and damage to bone marrow have been traced to long exposure to high concentration of oil (Mishra et al., 2001).

Polycyclic Aromatic Hydrocarbons (PAHs) are of particular concern in environmental pollution. They exhibit toxic, mutagenic and carcinogenic properties as they accumulate in food chains (Menzie et al., 1992). They often bioaccumulate in aquatic organisms (Nikunen et al., 2000). Oil spills is also responsible for film formation on shore lines, causing physical and aesthetic adverse effects in the environment. Altogether, these effects result in land degradation and water pollution limiting land use and damaging the ecosystems (Pettersson et al., 2009). It is necessary therefore, that effective counter measure be put in place to tackle the problem of oil spills in the Niger Delta, Nigeria. Microorganisms in the environment attack and digest the oil and this is the basis for the emergent field of bioremediation. Bioremediation is one of the most rapidly growing areas of environmental microbiology, which has been used for cleaning up pollutants. This is because of its low cost, safety and its public acceptability (Grazyna et al., 2001).

Fungi and bacteria have been found to be useful in bioremediation process. Members of the white rot basidiomycetous fungi are one group of organisms which extensively mineralize the recalcitrant PAHs due to their ability to produce lignolytic enzymes (Haritash and Kaushik, 2009). Amongst the diversity of microorganisms responsible for degradation of pollutants, Pseudomonas aeruginosa and P. fluorescens have the capability to degrade many different pollutants (Wackett, 2003).

The main objective of this study is to isolate and identify bacteria and fungi associated with crude oil-polluted areas in selected sites of Niger Delta, Nigeria using traditional techniques.

MATERIALS AND METHODS
Sources and collection of samples: The sampling sites for the oil-polluted soil and water were Awoye, Mese and Oluwa villages in Ondo State and three different flow stations (Agbada-Aluu shell, Obite and Bonny) in River State. The water samples were collected aseptically into screw-capped containers while the soil samples were collected into sterile cellophane bags. Uncontaminated samples were used as control.

Microbial analyses
Determination of bacterial and fungal populations: Pour plate technique was used for the microbiological analysis of samples collected from oil polluted sites (Song and Bartha, 1990). One gram of the soil and 1 mL of the water sample was mixed with 9 mL of sterile distilled water. One millilitre of the soil and water each was diluted from $10^{-1}$ to $10^{-10}$. An aliquot of (1 mL) dilution $10^{-4}$ was seeded into Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. The inoculated nutrient agar plates were incubated at 37°C for 24 h while the PDA plates were left at 28°C for 72 h (Amund and Igiri, 1990). The bacterial colonies and fungal spore forming units were then counted.

Purification and characterization of bacterial isolates: The streak method was used to subculture the bacterial isolates that grew on the NA. An inoculating loop was sterilized using hot flame, allowed to cool before it was used to take part of a grown bacterial colonies from the cultured agar and streaked on the surface of a fresh NA. The agar was incubated at 37°C in an inverted
position for 24-48 h. Individual bacterial colonies were identified by morphological and biochemical techniques using the taxonony scheme of Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994). The cultural characterization of bacteria colonies isolated was done by observing the colonies for colour, shape, edge, elevation and surface appearance displayed on NA.

**Gram staining:** Gram staining was done as described by Balows (1992). Single colonies obtained from the streaked plates were picked for the Gram staining. A drop of sterile water was placed on a clean, grease free slide and a smear of each colony was made with the water on the slide. This was heat fixed by passing the slide over a flame. A few drops of crystal violet was added to the heat fixed smear for 60 sec and rinsed with tap water. It was flooded with Gram’s iodine for 60 sec and rinsed with tap water. The smear was decolorized with alcohol until there was no more stain and rinsed with running tap water. Safranin was added to the smear for 40 sec to counter stain and washed with tap water. The smear was dried with soft paper. Immersion oil was placed on the smear and observed under the microscope at x100. The shape, arrangement and colour of the cells were examined. Gram positive bacteria stained purple while the Gram negative bacteria stained pink.

**Motility test:** Semi-solid nutrient agar was prepared in a tube and autoclaved. It was inoculated with each bacterial isolate and incubated at 28±2°C for 24 h. Bacteria which grew along the straight line of streak were described as being non-motile. Bacteria which grew away from the line or through out the medium were motile.

**Spore stain:** This is to detect the presence of bacterial endospores in isolates. A smear of each bacterial isolate was made on a clean slide. The smear was covered with a saturated aqueous solution of malachite green and steamed for 5 min. The slide was then rinsed in running water and counter stained with safranin solution for 1 min, rinsed in tap water and blotted dry between filter paper. It was then observed under the microscope with oil immersion. Spores when present are stained green in colour while the vegetative cells are stained red.

**Biochemical tests**

**Catalase test:** Catalase is an enzyme found in most bacteria. It catalyses the breakdown of hydrogen peroxide with the release of free oxygen and water. The test is used to determine whether a bacterium can produce the catalase enzyme. A loopfull of 24 h old culture of each isolate was put on a clean slide. A drop of 3% H₂O₂ was added to it. The production of bubbles showed the presence of catalase enzyme.

**Starch hydrolysis test:** This was done to check for the ability of the bacterial isolates to utilize starch. Nutrient agar containing 1% starch powder was prepared sterilized in an autoclave at 120°C for 15 min. The agar was allowed to cool to about 45°C before pouring into the plates and allowed to gel. Then bacterial isolates were inoculated on the agar. The plates were then incubated at 37°C for 24 h after which Lugol’s iodine was spread on the agar. The colour around the bacterial colonies was observed. The colonies that were blue-black showed that starch was not hydrolysed, while those that turned brown or yellow coloration showed hydrolysis of starch.

**Urease test:** Urease is an enzyme that acts on urea. Urease test was therefore done to test for the ability of bacteria to produce urease. Urea Agar Base (UAB) was used and it was prepared by
dissolving 2.1 g of the powder in 100 mL of distilled water. The mixture was heated to boiling on a hot plate. The boiled UAB was poured into various McCartney bottles and autoclaved at 121°C for 15 min. The bottles were slanted to make agar slants. The bacterial isolates were inoculated onto the slants. The slants were then incubated for a week at 37°C. Change of colour from light pink to light yellow indicated production of urease.

**Determination of sugar fermentation:** This is important in differentiating not only the genera, but species of bacteria. It shows the ability of bacteria to breakdown some sugars. Peptone (2 g), 1 g each (glucose, lactose, sucrose, mannitol, xylose and arabinose), 0.1 g NaCl and 0.1 g phenol red indicator were dissolved in 200 mL of distilled water. The mixture was heated on a hot plate for complete dissolusion of the ingredient. Five milliliters was pipetted into each test tube. Durham's tube was added to each tube in an inverted position and filled up with the medium and autoclaved at 121°C. The medium was inoculated for 15 min with each isolate in separate tubes and incubated at 37°C for 1-3 days. Change in colour from red to yellow indicated the ability to ferment. Creation of empty space in the closed end of the Durham's tube in the medium indicated production of gas.

**Acid fast test:** A heat fixed smear of 24 h grown culture of each bacterium was prepared on a glass slide. The smear was heated on a hot plate at 80°C for 15 min. Then the smear was flooded with carbon fuchsin for 5 min. It was rinsed with deionized water and tilted to drain. The smear was decolorized with acid alcohol (95% ethanol and 3% hydrochloric acid) for 3 min. It was rinsed with deionized water and drained. The smear was flooded with methylene blue for 1 min, rinsed with deionized water and allowed to air dry. This smear was covered with oil immersion and examined under the microscope at x100 for the presence or absence of red cells. The cells that retained red stain were acid fast bacteria.

**Determination of nitrate reduction:** Nitrate is reduced by some microorganisms to nitrite, ammonia or free nitrogen. Tubes of nitrate broth (5 mL) were prepared with 0.1% potassium nitrate, peptone and yeast extract. After the test tubes and their contents had been sterilized and cooled, inoculum was added to the medium and incubated for 3 days at 37°C. The presence of gas in the Durham's tube indicated production of nitrogen gas.

**Oxidase test:** Presence of cytochrome C in microorganisms means that the organism produces a positive oxidase test. Culture was streaked on the surface of solidified Nutrient Agar (NA) and incubated at 37°C for 24 h until a growth was observed. One percent of aqueous solution of tetramethyl-P-phenylenediamine hydrogen chloride was poured over the surface of the grown culture. Oxidase positive colony developed a pink colour which became successively dark red, purple and black within 10-30 min.

**Statistical analysis:** The statistical analysis was performed using Microsoft office Excel 2007 for calculating mean, standard deviation and standard error.

**RESULTS AND DISCUSSION**

**Microbial population of environmental samples from oil-polluted sites:** Bacterial counts (x10^9) of oil-polluted soil and water collected from Ondo and River States are presented in Fig. 1. The bacterial loads of the soil ranged between 0±0 CFU g⁻¹ in Mese and 31.67±3.2 CFU g⁻¹ (Bonny flow station). The bacterial load of the Obite water was highest (33.00±1.0 CFU mL⁻¹) and
that of the Oluwa polluted water was lowest (10±0 CFU mL⁻¹). The sample collected from unpolluted soil and water (control) had bacterial counts of 7.33±2.5 and 10.33±3.5 CFU g⁻¹, respectively. These populations were lower than those of polluted sites.

Fungal loads (×10⁶) obtained from oil-polluted soil and water collected from Ondo and River States are presented in Fig. 2. Soil samples collected from Mese, Oluwa and Awoye had fungal counts of SFU g⁻¹, 4±2 and 16±3 SFU g⁻¹, respectively while the fungal population of the water
samples varied from 8.67±2.1 SFU mL⁻¹ (Oluwa) to 15.00±1.0 SFU mL⁻¹ (Awoye). The fungal counts for the soil and water samples collected from Agbada-Aluu, Obite and Bonny samples were higher (20.67±1.5-26.67±2.5 SFU g⁻¹ for soil and 23±0-25.67±2.1 SFU mL⁻¹ for water) than those of Mese, Oluwa and Awoye villages.

The microbial population obtained from this study shows that bacterial counts were higher than fungi counts. This corroborates the findings of Onifade and Abubakar (2007) and Olukunle et al. (2012) and this could be attributed to the nutrients status of the soils and water as suggested by Jobson et al. (1979).

Cultural, microscopic and biochemical characteristics of bacteria and fungi isolated from oil-polluted sites

Bacteria: The morphological and biochemical characteristics of bacteria isolated from polluted water and soil samples of Mese, Awoye and Oluwa in Ondo State are presented in Table 1. These include Bacillus firmus, B. sphaericus, Staphylococcus aureus, Micrococcus sp. Actinobacter sp., Pseudomonas stutzeri and B. pumilus. The bacteria isolated from Agbada-Aluu, Obite and Bonny in River State include B. megaterium, B. subtilis, B. pumilus, Micrococcus varians, Aerococcus viridans, Cellulomonas flavigen and Corynebacterium sp. (Table 2). The genera of Pseudomonas, Bacillus and Micrococcus isolated and identified in this study were also identified by Boboye et al. (2010), who worked on degradative activity of bacteria obtained from crude oil polluted sites. The genera of Micrococcus, Aerococcus, Clostridium, Staphylococcus, Streptococcus, Lactobacillus, Bacillus, Cellulomonas and Corynebacterium are gram-positive while species in the genera Enterobacter, Pseudomonas and Actinobacter are gram-negative. The spore formers include Clostridium sporogenes, Lactobacillus acidophilus, Corynebacterium sp. and Bacillus sp. In a study carried out to isolate crude oil-degrading marine bacteria by Sakalle and Rajkumar (2009),

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Isolate A</th>
<th>Isolate B</th>
<th>Isolate C</th>
<th>Isolate D</th>
<th>Isolate E</th>
<th>Isolate F</th>
<th>Isolate G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultural</td>
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<td></td>
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<td></td>
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<tr>
<td>Colony shape</td>
<td>Circular</td>
<td>Irregular</td>
<td>Irregular</td>
<td>Circular</td>
<td>Oval</td>
<td>Circular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Elevation</td>
<td>Raised</td>
<td>Flat</td>
<td>Flat</td>
<td>Raised</td>
<td>raised</td>
<td>Flat</td>
<td></td>
</tr>
<tr>
<td>Edge</td>
<td>Entire</td>
<td>Undulate</td>
<td>Entire</td>
<td>Entire</td>
<td>Entire</td>
<td>Undulate</td>
<td></td>
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<tr>
<td>Pigmentation</td>
<td>White</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Cream</td>
<td>Cream</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>Gram stain</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td></td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Spherical</td>
<td>Rod</td>
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<td>Rod</td>
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<tr>
<td>Spore stain</td>
<td>+</td>
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<td>-</td>
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<td>+</td>
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<tr>
<td>Catalase</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
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<td>Oxidase</td>
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<td>Fermentation</td>
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<td>Glucose</td>
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<tr>
<td>Mannitol</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Xylose</td>
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<td>+</td>
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<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Probable bacteria</td>
<td>Bacillus</td>
<td>Bacillus</td>
<td>Staphylococcus</td>
<td>Micrococcus</td>
<td>Actinobacter</td>
<td>Pseudomonas</td>
<td>Bacillus</td>
</tr>
<tr>
<td></td>
<td>firmus</td>
<td>sphaericus</td>
<td>aureus</td>
<td>sp.</td>
<td>sp.</td>
<td>stutzeri</td>
<td>pumilus</td>
</tr>
</tbody>
</table>

+ : Present, - : Absent
the bacteria obtained from a ship yard at Alang coast, Gujarat, India were species of genera *Acinetobacter*, *Marinococcus*, *Micrococcus*, *Planococcus*, *Methylbacterium*, *Rhodococcus* and *Noccardia*. These bacteria are at variant with what was obtained in this study with the exception of *Micrococcus. Pseudomonas* species, which is the most extensively studied among oil degraders as reported by Wackett (2003), was also detected in this study. These bacteria have the ability to degrade so many different contaminants.

**Fungi:** The macroscopic and cultural characteristics of fungi isolated from oil-polluted samples in Ondo and River States are presented in Table 3. The fungi appeared in various colours (yellow, black, brown, white and green) on Potato Dextrose Agar (PDA). The fungi identified were as follows: *Penicillium italicum*, *Aspergillus niger*, *P. oxalicum*, *Streptothrix atra*, *Articulosporum inflata*, *Gentriculosporium serpens*, *A. flavus*, *Halosprangium pavum*, *A. fumigatus* and *A. rapens*. The following fungi appeared brown on PDA: *G. serpens*, *Haplosporangium* spp., *S. atra* and *A. fumigatus* while *L. penicilloides* and *H. pavum* appeared green. On the other hand, *A. rapens*, *P. oxalicum* and *P. italicum* were yellowish green in colour on PDA while *A. niger* appeared black. The conidiophores of *P. italicum* were seen to arise from the mycelium singly near the apex while in *P. oxalicum*, the hyphae bore conidiophores. The conidiophores of *A. niger* were upright and terminated in a globose swelling and bore phialides at the apex. Those of *A. fumigatus* were smooth walled with flask shaped while the septate hyphae of *A. flavus* bore the conidiophores.
Table 3: Characteristics of fungi isolated from oil-polluted environmental samples in Ondo and river states

<table>
<thead>
<tr>
<th>Isolate cultural appearance</th>
<th>Microscopic features</th>
<th>Name of probable organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark brown filamentous</td>
<td>Conidiophores were erect with slender branches resulting from lower portion giving rise to sub branches</td>
<td>Geniculosporangium serpens</td>
</tr>
<tr>
<td>Pale brown hyphae</td>
<td>Conidiophore was single and pale near the apex. Conidia were pale brown and mostly lateral brown</td>
<td>Pleurophragmium sp.</td>
</tr>
<tr>
<td>Brown mycelium</td>
<td>Slender mycelium, giving rise to tapering conidiophore each bearing a conidium</td>
<td>Haplosporangium sp.</td>
</tr>
<tr>
<td>Yellowish green to dark green hyphae</td>
<td>Conidiophores arranged singly from the mycelium. They were mostly ovoid or globose near the apex</td>
<td>Penicillium italicum</td>
</tr>
<tr>
<td>Black mycelium</td>
<td>Conidiophores were upright, unbranched, simple and terminating in a globose swelling, bore phialides at the apex</td>
<td>Aperilllus niger</td>
</tr>
<tr>
<td>Greenish mycelium</td>
<td>It appeared as a long slender septate branch for many hyphae which bore simple conidiophore</td>
<td>Lagellopsora penicillidioides</td>
</tr>
<tr>
<td>Yellowish green coloured hyphae, Yellowish base</td>
<td>Septate hypha, hyphae were borne and conidiophores. Primary and secondary stigmata were present</td>
<td>Penicillium exallicum</td>
</tr>
<tr>
<td>Brown with creamy colour at the edges</td>
<td>Branched conidiophores with conidia. Dark mycelium growing loosely with spirally coiled conidiophores</td>
<td>Streptothrix atra</td>
</tr>
<tr>
<td>White, dense and compact mycelium</td>
<td>Hyaline conidiophore, slender with upper part bearing from branches</td>
<td>Articulosporium inflata</td>
</tr>
<tr>
<td>White initially and later changed to black</td>
<td>Septate hyphae bearing conidiophores</td>
<td>Asperillus flavus</td>
</tr>
<tr>
<td>Greenish mycelium</td>
<td>Presence of hyphae</td>
<td>Halosporangium pavum</td>
</tr>
<tr>
<td>Brown mycelium</td>
<td>Conidiophores were smooth walled with flask shape. Vesicle with flask shape series of stigmata was produced. There were parallel rows of conidia</td>
<td>Asperillus fumigatus</td>
</tr>
<tr>
<td>Yellow mycelium with black spores</td>
<td>Septate hyphae</td>
<td>Asperillus rapens</td>
</tr>
<tr>
<td>White mycelia</td>
<td>Conidia arthropore were hyaline. J. celled shut cylindrical with truncate end</td>
<td>Geotrichum albidum</td>
</tr>
</tbody>
</table>

Some of the fungi are similar to those identified in a previous study that was conducted to assay for degrading activity of fungi on kerosene, diesel and petrol using classical selective enrichment method Olukunle et al. (2011).

Only the species of the general of Micrococcus and Bacillus were found in all the water samples. Bacillus pumilus, B. subtilis, B. megaterium and Micrococcus varians occurred in soil and water collected from Obite. Bacillus subtilis was found in all the samples collected from River State.

H. parvum and A. rapens were common in soil and water in Agbada-Aluu, Obite and Bonny flow stations samples from River States but not in Ondo States samples. In contrast, Streptothrix atra and A. niger were present in all soil and water samples of oil-polluted sites in Ondo States safe that the later mold was absent in Mese water. P. italicum and A. niger occurred in all the water and soil samples, respectively.
Microbiological study carried out on the environmental samples revealed the presence of both bacteria and fungi in the soil and water tested. This confirms that microorganisms are ubiquitous, diverse in nature and are able to adapt in extreme environments (Watanabe et al., 2002). Oil-polluted sites do harbour a vast array of microbial flora, hence diversity in the types of bacteria and fungi identified in this study. The existence of these microorganisms in the oil polluted environment suggests that the isolated bacteria could utilize the oil as energy and carbon source (Das and Mukherjee, 2007). The high loads of microbes indicate that the indigenous microbes in the polluted soil and water have catabolized part of the oil and used it to grow. According to Ojo (2009), the activities of the indigenous microbes could be responsible for the bioremediation of the polluted sites. The unpolluted samples had the least population of bacteria and fungi in this study. This low population means that natural unpolluted water and soil environment do not contain abundant microorganisms because there was no carbon and energy source in the environment that could encourage them to multiply. Similar reports were published by Okerentugba and Ezeronye (2008), Onifade and Abubakar (2007) and Boboye et al. (2010). According to them, high numbers of certain oil-degrading microorganisms from an environment implies that those organisms are the active degraders of that oil. In particular, the proportion of microbial populations with plasmids containing genes for utilization are increased (Atlas, 1995). Certain indigenous microorganisms have been shown to have the capability to degrade crude oil in polluted soils leading to in situ bioremediation. In situ bioremediation provides an effective, economical, versatile and environmental friendly means of reclaiming polluted lands.

Identification of environmental bacteria is often complex to analyze using traditional techniques because of the uncultured bacteria that cannot easily be identified by these techniques (Kamagata and Tamaki, 2005). It is reported that only about 1% bacteria can be detected from many environments when traditional techniques are used (Amann et al., 1995; Pace, 1997; Kamagata and Tamaki, 2005).

As a result of the limitations associated with traditional techniques, new strategies and approaches are being implemented for the rapid, sensitive and specific detection of microorganisms in the environment. The molecular analyses have been found to be more appropriate than the traditional approaches. However, both molecular and traditional techniques are important to characterize novel organisms for bioremediation. However, further research to assay for the degradative ability of the microorganisms is necessary for effective and efficient ex situ bioremediation process.

CONCLUSION

The results obtained in this study have revealed that oil-degrading bacteria and fungi can be isolated from oil polluted sites. These bacteria and fungi are indigenous in the polluted sites and they are responsible for the degradation of oil. The traditional techniques of identification, however, enables microbiologist to understand the basic principle of identification of microorganisms which are based on morphological, metabolic and physiologic traits. Traditional techniques are useful especially in laboratories where there are no molecular biology facilities. To make bioremediation effective, screening for microorganisms using both techniques are necessary so as to obtain indigenous novel organisms.

REFERENCES


