Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.3, No.13, 2013

Microbial Population and Physicochemical Properties of Oil-polluted sites in selected areas of Niger Delta, Nigeria

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Abstract

Oil spills are known to be the most important and widespread forms of pollution of agricultural lands and water bodies in the Niger Delta regions in Nigeria, due to the increasing demand for energy. This is an inevitable disaster which has to be attended to, in order to minimize its harmful effect on the ecosystem, thereby, conserving nature and sustaining livelihood. The aims of this study are to determine the growth of the indigenous bacteria and fungi from oil-polluted areas in Niger Delta, on kerosene, petrol and diesel and determine the physicochemical properties of the oil-polluted soils and waters bodies. Pour plate technique was employed for the enumeration of bacteria and fungi using Bushnell-Haas medium and Minimal Salt medium respectively. The culture media were supplemented with 2% oil (kerosene, diesel, and petrol) as sole source of carbon and energy. Physicochemical parameters of the environmental samples were analyzed on the environmental samples using standard chemical methods such as oven-drying method, EDTA titrimetric method and atomic absorption spectrophotometry. The growth of indigenous bacteria and fungi on the different oils confirms their degradative ability. Hence, they are responsible for the degrading activity in the sites where the samples were collected. The physicochemical parameters obtained in this study revealed that the moisture contents and pH of the samples were optimum for oil degradation. The oil and grease found in the water samples show that the samples were polluted with oil and grease. The concentrations of phosphates and nitrates were low while the concentration of lead was high when compared with the recommended standard. In conclusion, the physicochemical analyses of the soil and water showed that the soils are not good for agriculture and the water, not good for consumption. This calls for immediate bioremediation of the affected areas using biostimulation.

Keywords: Microbial population, biostimulation, physicochemical parameters, degrading ability.

1. Introduction

Oil spillage is known to be a major environmental problem in Nigeria, most especially in the Niger-Delta. It is reported that oil spillage has caused constant threat to farmlands, crop plants and forest tree species (Ogri, 2001; Agbogidi, 2003). It destroys soil fertility, causes alterations in soil physicochemical and microbiological properties, thereby having detrimental effects on the terrestrial and aquatic ecosystems. Oil spills also cause epidemics of many diseases because spilled oils contain toxic substances (Nwachukwu *et al*, 1999), which could be injurious to human health. The indirect effects of oil spills in soil include oxygen deprivation of plant roots as a result of exhaustion of the soil oxygen by oil-degrading microorganisms, which create anaerobic conditions that may lead to the formation of hydrogen sulphide (Agbogidi *et al.*, 2005). The direct effect on the ecosystem includes damage of fur and feathers of birds, making them prone to death by freezing. As a result of these effects on the ecosystem, the release of oil into the environment has caused serious environmental concern and attracts public attention (Roling *et al*., 2002). In order to reduce or eliminate the effect of oil spillage on the environment and living organisms, physical, chemical and biological methods have been employed. Efforts such as application of chemical dispersants, skimming of the surface oils, application of biological oil agents and inoculating the spilled areas with relevant microbes are the outcomes of intensive research. The most promising of many researches carried out to deal with large-scale oil spills is the use of microorganisms to provide an effective alternative (Singh *et al.*, 2001). This approach is referred to as bioremediation. Okon and Hernandez, (2006) defined bioremediation as any process that uses microorganisms or their enzymes to remediate the environment altered by contaminants. Dua *et al*. (2002) reported that microorganisms are capable of using organic substances, natural or synthetic, as sources of nutrients and energy hence, exhibiting remarkable range of degradative capabilities. 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).

Temperature, pH, adequate inorganic nutrients and relative humidity of the environment are factors that affect the growth of microorganisms responsible for oil degradation (Dubey, 2009). These microorganisms derive nutrients and energy for optimal growth and reproduction from the oils so as to utilize or degrade them. Biodegradation, which is the destruction of organic compounds by microorganisms, is carried out largely by

diverse bacterial populations, mostly *Pseudomonas* species. Although, most organisms are endowed with detoxification abilities, i.e. mineralization, transformation and/or immobilization of pollutants, microorganisms, particularly bacteria, have been the well-studied and are the most frequently used for bioremediation strategies (Diaz, 2004). *Cladophialophora* sp. was found to have the ability to degrade toluene, ethylbenzene, and xylene (Prenafeta-Boldú *et al*., 2002) while *Cyclothyrium* sp. was found to be efficient in the degradation of pyrene, phenanthrene, anthracene, and benzo[a]pyrene (Da Silver *et al*., 2003). Members of white rot basidiomycetous fungi have been reported as one group of organisms which extensively mineralize the recalcitrant polycyclic aromatic hydrocarbons due to their abilities to produce ligninolytic enzymes (Pointing, 2001). Certain white rot fungi such as the *Pluerotus* sp., *Phanerochaete chrysosporium*, *Phanerochaete laevis*, *Trametes versicolor* (Pointing, 2001) and *Agrocybe* sp. (Chupungars *et al*., 2009) have been well recognized for their capability to degrade polycyclic aromatic hydrocarbons. However, the consortia proved to be a better degrader compared to individual isolates (Ghazali e*t al.*, 2004; Gerdes *et al*., 2004; Trindade *et al*., 2004: Sun *et al*., 2005: Mandri and Lin 2007).

The ability of an organism to degrade a compound depends on the ability of the compound to come into contact with an enzyme or a series of enzymes which can degrade it. The principle of degradation involves: accessibility of the compound to the enzymes, ability of the enzymes to degrade the compound and production of large quantity of the enzyme to carry out the degradation process.

The aims of this study are to determine the growth of the indigenous bacteria and fungi from oil-polluted areas in Niger Delta, on kerosene, petrol and diesel and determine the physicochemical properties of the oil-polluted soils and waters bodies.

2. Materials and Methods

2.1. Sources and collection of samples

The sampling sites for the oil-contaminated environmental samples (water and soil) were Awoye, Mese and Oluwa villages in Ondo State; and three different flow stations (Agbada-Aluu shell, Obite, and Bonny) in Rivers State. The water samples were collected aseptically into screw-capped containers while the soil samples were collected into sterile cellophane bags. Uncontaminated samples were collected and used as control.

2.2. Microbial analysis

2.2.1. Enumeration of bacteria and fungi

Pour plate technique (BHA) was employed for the enumeration of bacteria (Bushnell-Haas, 1941; Atlas, 1994). After sterilization of the enrichment medium at 121^{o}C for 15 min, it was supplemented with 2% (v/v) filter sterilized oils (paraffin, petrol and diesel) separately to serve as the only source of carbon (Ijah and Abioye, 2003). The soil (g) and water (ml) samples were serially diluted and 1mL suspension was aseptically transferred from each 10^3 dilution into sterile Petri dish and seeded with BHA using pour plate technique. The medium was allowed to gel and incubated at 30^{o}C for 1 - 3 days. A control devoid of the sample was prepared for each set of the experiments. All experiments were performed in triplicate. After incubation, the colonies that grew on the agar were counted.

2.2.2 Enumeration of oil-degrading fungi

The minimal salt medium (MSM) of Zajic and Supplison as described by ljah and Abioye (2003) was used. After autoclaving the medium, it was supplemented with 2% (v/v) filter sterilized oils (kerosene, diesel and petrol) to serve as the only source of carbon and energy (Ijah and Abioye, 2003). One millilitre from $l0^{-l}$ to 10^{-6} serially diluted suspension of soil and water samples were seeded in the MSM agar using the method of Atlas and Bartha (1981). The agar was incubated at 28 ± 2°C for 3 – 7 days.

2.2.3 Physicochemical analysis

The pH of the samples was measured with the aid of electric pH meter (Jenway 3510) pH meter after calibrating it with buffer solutions of pH 4, 7 and 10. The moisture content of the soil was determined using oven-drying method, which is based on weight loss of water due to evaporation. The total nitrogen and available phosphorus were determined by standard titrimetric procedures (A.O.A.C, 2003). Exchangeable Mg and Ca were determined by EDTA titrimetric method while exchangeable Na and K in the soils were determined using flame emission photometry. Atomic absorption spectrophotometry (AAS) was used to analyze for the heavy metals such as Pb, Zn, Fe, Cu, Cr, and Ni in the soil and water samples. The BOD was analyzed by the method based on biological oxidation of organic materials by aerobic bacteria while COD was analyzed by the method based on chemical oxidation of materials in the presence of catalyst by $Cr_2O_7^{2-}$ in 50% H_2SO_4 as described by De (1999).

3. Results

3.1. Bacterial population in soil

The results showing the load of bacteria associated with oil-polluted soil when grown on BHA supplemented with kerosene, diesel and petrol are presented in Figure 1. The population of oil-degrading bacteria that grew on kerosene, diesel and petrol ranged between 17.67 ± 3.5 Cfu/g (Mese and Agbada-Aluu soils) and 24.00 ± 1.0

Cfu/g (Bonny soil); 16.67± 2.5 Cfu/g (Agbada-Aluu soil) and 22.67± 1.5 Cfu/g (Bonny soil) and 21.33 ± 1.5 Cfu/g (Agbada-Aluu soil) and 27.00 ± 2 Cfu/g (Obite soil) respectively. The bacterial population obtained from unpolluted soil samples contained fewer bacteria than the oil spilled soil when grown on the various oils.

3.2. Bacterial population in water

The bacteria isolated from water samples when grown on kerosene, diesel and petrol had the populations of 13.67 ±2.5 Cfu/mL (Mese water) and 21 ± 2.6 Cfu/mL (Agbada-Aluu water) on kerosene; 17.00 ± 1.0 Cfu/mL (Mese water) and 23.00 ± 2.0 Cfu/mL (Bonny water) on diesel; and 19.67± 2.1 Cfu/mL (Mese and Awoye) and 23.67 ± 1.5 Cfu/mL (Agbada-Aluu) on petrol (Fig. 2). Relatively, the bacteria grew better on petrol than diesel and kerosene. However, the bacterial population on kerosene being the lowest is higher than those of unpolluted soil and water.

3.3. Fungal population in soil

The population of fungi obtained from oil-polluted soil when grown on minimal salt medium (MSM) supplemented with kerosene, diesel and petrol are presented in Figure 3. Similar to the loads of bacteria on soils, the fungi from soils grew best in petrol (20.00 ± 1.0 Sfu/g (Awoye) to 25.67 ± 1.2 Sfu/g (Oluwa) than kerosene (16.00 ± 2.0 Sfu/g (Bonny) to (30.33 ± 1.5 Sfu/g (Obite); and diesel except for samples from Agbada-Aluu and Obite (Fig. 1). There is no considerable difference in the fungi population of what grew from Mese soils in diesel and petrol.

3.4 Fungal population in water

The lowest fungal population from oil polluted water that survived on kerosene, diesel and petrol were 18.00 ± 2.0 Sfu/mL (Awoye); 19.33 ± 1.5 Sfu/mL (Oluwa) and 17.00 ± 2.0 Sfu/mL (Obite) respectively. The highest fungal populations observed were 22.33 ±1.5 Sfu/mL (Mese); 23.67±1.5 (Bonny) and 25.33 ± 2.1 (Awoye). The unpolluted water samples contained fewer fungi than in the oil spilled water (Fig. 4).

3.5 **Physicochemical properties of oil polluted soil**

The physicochemical properties of oil polluted soil collected from Ondo and Rivers States are presented in Table 1. The analyses of the soils from Awoye, Mese, Oluwa, Agbada-Aluu, Obite and Bonny revealed that the moisture contents of the oil polluted sites ranged from 61.51% to 79.85%. The pH values ranged from 3.9 to 6.7. The highest and lowest values were obtained from Mese and Agbada-Aluu soils respectively. It was observed that the lowest concentrations of Mg, Na, K, PO_4, and N_2 were found in oil polluted Obite soil while the highest concentrations were obtained in Awoye soil except for Mg that has the highest concentration in Mese and Awoye soils. The values for the organic matter ranged between 0.69 for Bonny and 5.88 for Awoye soils. Awoye soils was found to contain the highest amounts (4.23%) and (5.88%) respectively of organic carbon and organic matter.

3.6. Physicochemical properties of oil polluted water

The physicochemical analyses of the oil polluted water from Oluwa village had the highest pH (6.8) followed by water samples from Awoye and Mese villages with pH values 6.7 and 6.6 respectively. The pH of the oil spilled water samples collected from the three flow stations was lower than those obtained for Mese, Oluwa and Awoye water samples. The biochemical oxygen demand (BOD) obtained in this study ranged from 30.7mg/l (Awoye) to 34.3mg/l (Bonny) while the chemical oxygen demand (COD) ranged between 40.1mg/l (Oluwa) and 42.7mg/l (Obite). The concentrations of NH_3 ranged from 15 mg/l (Mese) to 16.7 mg/l (Agbada-Aluu). The values for the electrical conductivity ranged from (1179.4 - 1198.3) µS/cm with highest value observed in the sample from Agbada-Aluu and the least value in sample from Mese. The oil and grease values ranged from (26.42 – 32.12) mg/l, with the highest values observed in the polluted soil from Mese and the least value in the samples from Obite. It was observed in all the oil polluted water samples that the concentration of the nitrates, copper and nickel were 0.01mg/l, 0.001mg/l, and 0.01mg/l respectively. The physicochemical parameters for the control sample are also contained in Tables 1 and 2.

4. Discussion

The considerable potentials of isolated bacteria and fungi grown on kerosene, diesel and petrol are shown in Figures 1 and 2 and figures 3 and 4 respectively. The degrading capabilities on different oils revealed that the microorganisms isolated from the soil and water samples were able to degrade oil. The cells were able to multiply during the incubation periods, indicating that they were able to degrade and utilize the oils for their growth and development. All the organisms maximally utilized all the hydrocarbon substrates (petrol, kerosene and diesel) when supplied as the sole source of carbon and energy. Although, the level of oil utilization differs from one microbe to another due to the differences in their metabolic rates; and from one type of hydrocarbon to the other due to the differences in the molecular sizes of the hydrocarbons.

The moisture contents of all the polluted soil samples are within the range that could support bioremediation. These values are in agreement with the findings of Bossert and Bartha (1984) where they concluded that moisture contents ranged between 20 – 80% are generally optimum for hydrocarbon degradation. In all the soil samples, the concentration of calcium, magnesium, potassium, sodium, phosphorus and nitrogen was lower than

what was observed in the unpolluted soil with the exception of Awoye and Oluwa samples. The findings in this study is in contrast to the work carried out by Wyszkowski *et al*. (2004) who reported higher concentrations of phosphorus, sodium, magnesium and calcium in above ground parts and roots of yellow lupine when the soil was polluted with diesel. The relative low concentrations of these elements in the polluted soil samples were due to their utilization by the indigenous microorganisms which led to their reduction. This result is similar to that of Okpokwasili, (2003). The low pH values of oil-polluted water and soils samples from Agbada-Aluu, Obite and Bonny indicate that they were highly acidic. The polluted samples from Awoye, Mese and Oluwa were slightly acidic. The pH values of the polluted samples obtained in this study were lower than those of polluted samples. These results are similar to the findings of Amadi *et al*. (1993) and Chukwuma *et al*. (2010), but differ from that of Isirimah *et al*. (1989). Previous studies had shown that low pH is toxic to fish and other aquatic lives (Baker, and Schofield, 1982).

COD is the amount of chemical oxidant required for the oxidation of organic matter present in the polluted samples while BOD is the amounts of oxygen required by the aerobic bacteria to biochemically oxidize the organic matter present in the polluted samples. The high values of BOD in all the samples suggest that the aerobic bacteria are oxidizing the oxygen present in the polluted samples. This may be responsible for the death of aquatic organisms. The normal range of BOD for good water quality is 5-6 mg/l and COD is 6-10 mg/l (Huq *et al.* 2005). Higher BOD and COD values obtained in this study indicate that the water is considerably polluted with organic and chemical pollutants.

In all the oil polluted samples, the data revealed that the concentrations of Zn, Fe and Pb were higher in the polluted water samples than the unpolluted sample while the concentrations in both the polluted and unpolluted water samples were the same for Cu, Cr and Ni. Higher electrical conductivity and salinity were also obtained for polluted water samples. The results are in agreement with that of Hardik, (2011), who reported high concentration of heavy metals in polluted samples. According to him, the greater electrical conductivity in polluted samples is attributed to the presence of metal ion. Also, higher electrical conductivity indicates high salinity. The high concentration of lead observed in the Obite samples may be responsible for the heavy metal associated diseases such as liver and kidney diseases, damage to bone marrow and cancer reported in Niger Delta by Lloyd and Cackette, (2001) and Mishra *et al*., (2001). The higher concentration of zinc in the polluted samples corroborates the findings of Chukwuma, (2010) who found that zinc was higher in crude oil polluted soils than in non-polluted soil.
The high concentration of heavy metals has also been reported to negatively affect crop growth. The higher concentration of salinity obtained for all the oil polluted samples compared with the unpolluted sample is similar to the findings of Otu, (2002), who also reported higher concentration of salinity on petroleum-hydrocarbon polluted sandy beach samples.

5. Conclusion

The growth of indigenous bacteria and fungi on the different oils confirms their degradative ability. Hence, they are responsible for the degrading activity in the sites. The physicochemical properties of the soil and water showed that the soils are not good for agriculture and the water, not good for consumption. This calls for immediate bioremediation of the affected areas. Biostimulation, which is the stimulation of the indigenous microorganisms by optimizing factors such as nutrients, oxygenation, temperature, pH, and addition of biosurfactants could be employed in the bioremediation strategy.

6. Acknowledgement

The author sincerely appreciates the Federal University of Technology (FUTA) for supporting this research and Dr . O. O. Fatoba, Department of Chemistry, University of the Westarn Cape, Cape Town for painstakingly reading through and correcting the manuscript.

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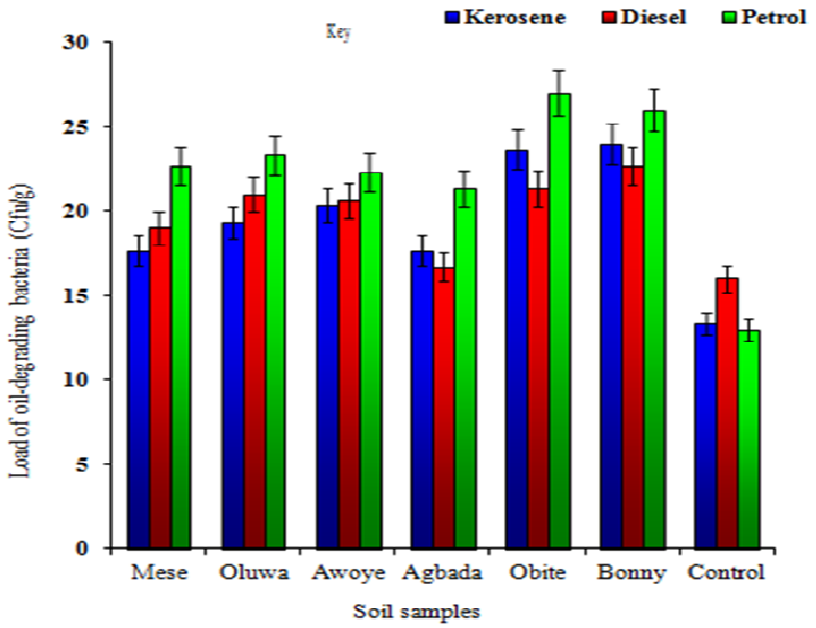

Figure 1: Effect of various oils on the population of bacteria isolated from oil-polluted soil

Legend:
Agbada Agbada-Aluu

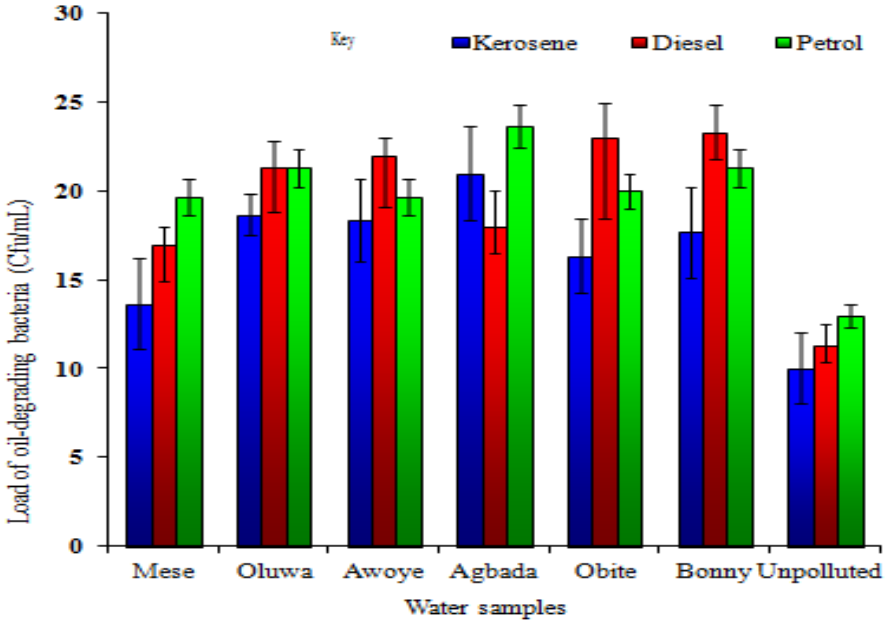

Figure 2: Effect of various oils on the population of bacteria isolated from oil-polluted water

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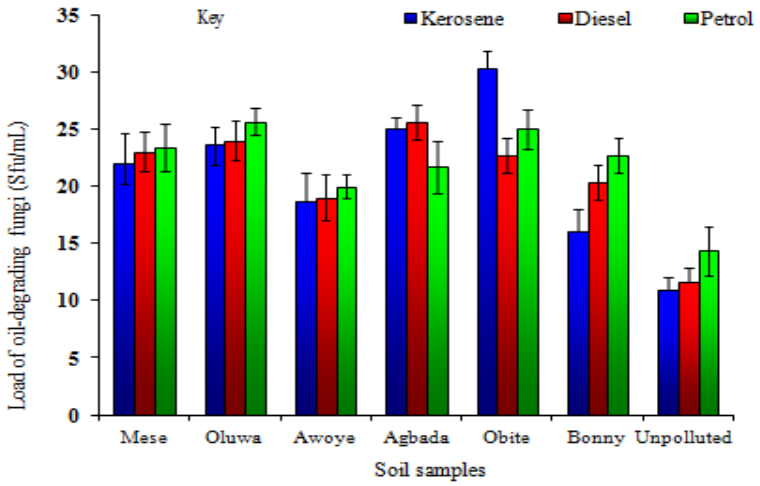

Figure 3: Population effect of various oils on fungi isolated from oil-polluted soil

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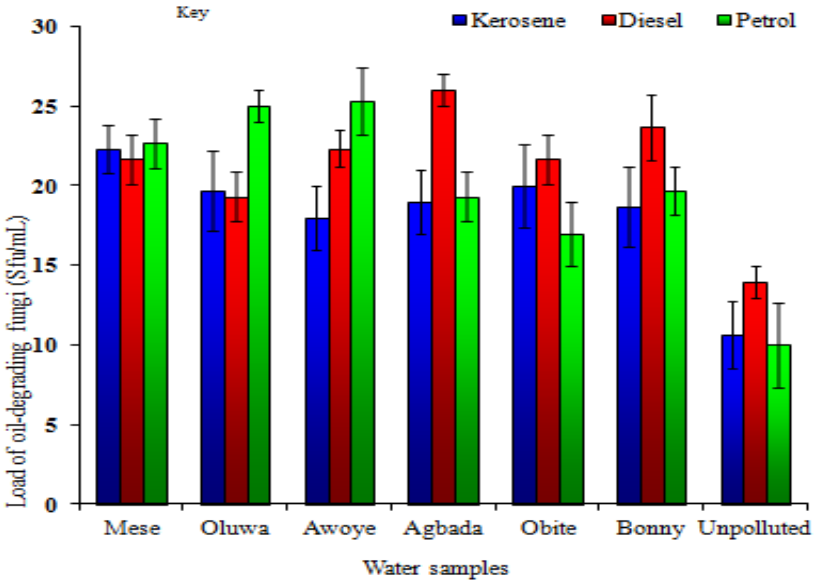

Figure 4: Population effect of various oils on fungi isolated from oil-polluted water

Legend:
Agbada Agbada-Aluu

Table 1: Physicochemical properties of oil polluted soil collected from Ondo and Rivers States

Parameters	Awoye	Mese	Oluwa	Agbada	Obite	Bonny	Unpolluted
Moisture content (%)	77.95 ± 1.3	78.65 ± 1.2	77.89 ± 1.5	79.85 ± 0.6	62.88 ± 0.5	61.51 ± 2.1	79.34 ± 1.2
pH	6.4 ± 0.09	6.7 ± 0.09	6.5 ± 0.03	3.9 ± 0.05	4.7 ± 0.04	4.6 ± 0.05	7.9 ± 0.09
Organic matter (%)	5.88 ± 1.8	4.75 ± 1.4	4.97 ± 1.6	1.29 ± 1.3	0.73 ± 1.2	0.69 ± 2.0	5.96 ± 1.7
Magnesium (mg/kg)	2.0 ± 2.3	2.7 ± 1.3	2.4 ± 1.6	0.91 ± 2.3	0.81 ± 2.1	0.89 ± 1.9	2.8 ± 1.4
Calcium (mg/kg)	5.8 ± 1.6	2.3 ± 1.4	4.8 ± 2.2	1.74 ± 3.3	1.14 ± 0.9	1.01 ± 1.2	4.0 ± 1.1
Sodium (mg/kg)	0.91 ± 0.8	0.73 ± 1.2	0.82 ± 3.2	0.421 ± 3.1	0.323 ± 2.3	0.329 ± 1.9	0.71 ± 1.4
Potassium (mg/kg)	1.15 ± 3.4	0.81 ± 1.3	1.02 ± 2.6	0.319 ± 2.5	0.206 ± 1.7	0.303 ± 1.0	1.74 ± 0.8
Phosphate (mg/kg)	5.51 ± 2.1	1.75 ± 1.1	2.34 ± 1.8	4.3 ± 1.8	1.20 ± 3.3	1.00 ± 3.1	5.88 ± 2.1
Nitrogen (%)	0.56 ± 1.1	0.42 ± 1.4	0.48 ± 2.2	0.4 ± 2.7	0.3 ± 2.1	0.3 ± 1.9	0.65 ± 1.6
Organic carbon (%)	3.41 ± 0.9	2.76 ± 1.0	3.01 ± 0.8	4.12 ± 1.1	4.08 ± 1.3	4.23 ± 0.8	2.00 ± 1.0

Legend:
Unpolluted control

Table 2: Physicochemical properties of oil polluted water collected from Ondo and Rivers States

Parameters	Awoye	Mese	Oluwa	Agbada	Obite	Bonny	Unpolluted
pH	6.7 ± 0.9	6.6 ± 0.4	6.8 ± 0.4	4.27 ± 0.2	3.47 ± 0.5	4.27 ± 0.9	7.7 ± 0.7
Conductivity	1188.9 ± 1.3	1179.4 ± 1.0	1197.3± 1.9	1198.3 ± 2.2	1189.6 ± 2.4	1187.6 ± 1.9	1102.5 ± 3.2
BOD (mg/l)	30.7 ± 3.3	30.2 ± 2.3	30.9 ±3.0	32.6 ± 2.4	31.5 ± 0.8	34.3 ± 1.1	23.2 ± 1.2
COD (mg/l)	40.5 ± 2.6	40.2 ± 2.1	40.1 ± 2.0	42.2 ± 1.3	42.7 ± 2.3	41.6 ± 3.2	30.2 ± 1.1
Oil and grease (mg/l)	29.98 ± 0.9	26.42 ± 1.0	30.10 ± 1.2	31.21 ± 1.7	32.12 ± 0.8	30.56 ± 1.9	9.2 ± 1.1
NH_3 (mg/l)	15.7 ± 0.7	15.0 ± 2.2	15.4 ± 2.4	16.7 ± 0.9	16.5 ± 2.1	16.2 ± 3.0	7.2 ± 2.2
Nitrates (mg/l)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Phosphates (mg/l)	0.11 ± 0.7	0.11 ± 1.9	0.01 ± 0.8	0.32 ± 2.1	0.23 ± 3.0	0.24 ± 2.1	0.03 ± 2.2
Salinity	19.59 ± 0.9	19.52 ± 3.2	19.50 ± 2.4	20.34 ± 3.2	20.76 ± 1.1	21.14 ± 0.9	19.2 ± 2.1
Lead (mg/l)	60 ± 0.7	60 ± 0.9	62 ± 0.2	68 ± 0.7	69 ± 1.1	60 ± 2.2	30 ± 1.8
Zinc (mg/l)	0.161 ± 1.1	0.162 ± 1.8	0.169 ± 0.9	0.165 ± 2.3	0.167 ± 1.2	0.170 ± 0.4	0.005 ± 1.4
Iron (mg/l)	0.212 ± 2.3	0.214 ± 1.1	0.218± 1.0	0.234 ± 2.3	0.222 ± 1.5	0.212 ± 1.5	0.01 ± 1.2
Copper (mg/l)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Chromium (mg/l)	0	0	0	0	0	0	0
Nickel (mg/l)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Legend:
Unpolluted control