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Mineral composition of engine oil-polluted soil after degradation of the oil by the white rot fungus, *Pleurotus florida* (Mont.) singer, an edible fungus

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ABSTRACT

The ability of the macro-fungus, Pleurotus florida, to degrade engine oil in contaminated soil was investigated with a view to ascertain its efficacy in reducing the toxicity of polluted ecosystem. Sterile soil samples (100 g) contained in polypropylene bags (12 cm diameter × 30 cm height) were contaminated with engine oil at 5, 10, 15, 20 and 25% v/w concentrations, inoculated with P. florida mycelium and incubated at 28 to 30°C for 60 days. Soil samples from the polluted soil were analyzed after the incubation period for the changes in mineral composition of the soil. At 5 and 25% engine oil concentration, all the mineral elements tested for, namely: phosphorus, magnesium, calcium and potassium showed significant changes in their composition when compared to their controls. Organic carbon increased with increasing oil concentration at 15% but later declined as the concentration of the pollutant increased from 20 to 25% engine oil concentration. The highest level of increase of carbon content was 7.07% at 15% engine oil pollution, followed by a low decrease from 6.70 to 6.48% at 20 and 25% engine oil in the soil, respectively. Statistical analysis revealed significant differences between the inoculated samples and their control, indicating degradation of the polluting oil. The results are discussed against the background of hydrocarbon pollution in the natural environment and the potentials of using P. florida in the intervention.

Key words: *Pleurotus florida*, mycoremediation, engine oil, minerals, pollution.

INTRODUCTION

Environmental pollution with petroleum and petrochemical products has become a problem to oil producing countries. Oil pollution comes from oil-well blow-outs, seepage, and deballasting operations, sale and use of petroleum products, pipeline overflow and breakage, and storage tank spills (Obire and Wemedo, 1996). Obire and Amusan (2003) reported deliberate discharge of oilfield waste water or effluent as a source of environmental pollution. Efforts to achieve biodegradation of oil products have involved bacteria and fungi, since they are the only biological agents which have the metabolic capacity of utilizing petroleum carbon for cell synthesis (Jobson et al., 1974). The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration, on environmental factors and on the biological state of the organisms at the time of the contamination. It has been shown that *Lentinus squarrosulus* Mont. mineralized soil contaminated with various concentrations of crude oil resulting in increased nutrient contents in treated soils after 6 months of incubation (Adenipekun and Fasidi, 2005). When crude oil is spilled, it undergoes physical and chemical weatherings (Atlas and Bartha, 1973) which involve spreading, evaporation and dissolution of higher components, emulsification, dispersion, oxidation and biodegradation. In effect, the chemical (mineral) composition of the soil is affected. For instance, according to Shukry et al. (2013), in petroleum, oil polluted soil especially at 2 and 3% crude oil. It was noted that, Na, Mg and Ca decreased while K increased in shoots of jojoba. In roots, Na and Ca increased; however, K and Mg decreased with increasing crude oil concentration in the soil.

Heavy metals, Cu, Mn, Cd and Pb increased in both shoot and root with increasing crude oil concentration; while, Zn decreased compared with the control. In soil, N and K decreased meanwhile Cu, Fe, Mn and Zn as well as organic matter increased with increasing crude. Fungi can grow under environmentally stressed conditions such as low pH and poor nutrient status where bacterial growth might be limited. At low levels of contamination of crude oil, cultivation of the soil without nutrient amendment is possible (Toogood, 1974).

The aim of this study was to investigate the changes in the mineral composition of the engine oil polluted soil after 60 days of incubation with *Pleurotus florida*.

MATERIALS AND METHODS

The fungus *P. florida* used for this study was obtained from Dr. I. A. Okwujiako, a Senior Lecturer and Mycologist in the Department of Biological sciences, Michael Okpara University of Agriculture, Umudike. The culture was subcultured in malt extract agar to get pure growing culture. Garden soil used was collected from Umuahia, Abia State, Nigeria. The engine oil was obtained from a fuel station in Umuahia, Abia State, while the straw used was collected from a rice farm in Bende Local Government Area of Abia State.

Preparation of culture medium

Malt Extract Agar (MEA) was prepared by dissolving 20 g of agar powder and 20 g of malt extract broth in 1000 ml of distilled water. The mixture was autoclaved at 121°C for 15 min. On cooling to 45°C, the medium was dispensed into 9-cm Petri dishes to gel.

Spawn production

Spawn was prepared following a modified method described by Senyah et al. (1989). The guinea corn (*Sorghum bicolor*) grains used were thoroughly washed with tap water and soaked overnight. They were dispensed into spawn bottles and autoclaved at 121°C for 1 h each day for three consecutive days. On cooling, the grains in each bottle were inoculated with four 9 mm mycelia discs taken from a 4-day-old agar culture of *P. florida* and incubated at 28±2°C for 14 days in darkness.

Fungal cultivation

A modified method of Baldrian et al. (2000) was employed. 100 g aliquots of sterilized soil were weighed into polypropylene bags (12 cm diameter × 30 cm high) before sterilization. Different concentrations (5, 10, 15, 20 and 25% v/w) of engine oil were added and mixed thoroughly and 30 g of rice straw were laid on the contaminated soil. Each bag was inoculated with 7 g of spawn of the test fungus and tied with masking tape. All the bags were incubated at 28±2°C for 60 days. Completely randomized design was used in the experiment. Experimental data collected were analyzed using analysis of variance (ANOVA) at $\rho < 0.05$ to ascertain the level of significant difference between the control and the inoculated samples.

RESULTS AND DISCUSSION

Figures 1, 2, 3 and 4 show changes in the mineral composition of the soil polluted with engine oil after 60 days of incubation. For phosphorus content of the soil (Figure 1), there was a significant increase at 5% engine oil in the soil from 87.26 (control value) to 127.6 $mgkg^{-1}$. At 25% engine oil contamination, there was a low increase from 74.4 (control value) to 101.16 $mgkg^{-1}$. Similar studies were done by Adenipekun and Isikhuemhen (2008), where the highest level of increase in phosphorus observed was 30.40 μgml^{-1} at 1% engine oil in the soil, and lowest level of increase was 12.32 μgml^{-1} at 40% engine oil in the soil. Figure 2 shows that the highest level of decrease of potassium found in the polluted soil was 0.364 $molkg^{-1}$ at 5% engine oil in the soil and lowest level of decrease observed was 0.267 $molkg^{-1}$ at 25% contamination, when compared to their controls at 5 and 25% contamination which were 0.569 and 0.446 $molkg^{-1}$, respectively. A similar trend was also observed for magnesium and calcium as shown in Figures 3 and 4, respectively. The highest levels of decrease of these mineral elements were from 5.95 to 4.22 $molkg^{-1}$ and from 11.8 to 9.8 $molkg^{-1}$, respectively at 5% engine oil in the soil; whereas, the lowest levels of decrease were from 3.25 to 2.83 mol kg^{-1} and from 9.0 to 7.8 mol kg^{-1}, respectively at 25% engine oil in the soil, when compared to their controls. Oil products when added to the soil create a very high carbon and nitrogen ratio (C:N), where the essential elements of nitrogen, potassium and phosphorus become the limiting factor to oil degradation by bacteria and fungi.

Benka-Coker and Ekundayo (1995) reported low levels of nitrogen and phosphorus from a crude oil spill site in the Niger Delta of Nigeria. Low values for phosphorus, potassium and nitrogen reserve in petroleum hydrocarbon contamination were reported by Lehtomaki and Niemela (1975). However, the effect of oil on microbial populations depends upon the chemical composition of the oil and on the species of microorganisms present. Populations of some microbes increase; typically, such microbes use the petroleum hydrocarbons as nutrients. As they seek the nutrients, they break down the hydrocarbons which now amend the soil. The same crude oil can favor different genera at different temperatures (Westlake, 1974). Fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria, and although, hydrocarbon degraders may be expected to be readily isolated from a petroleum oil- associated environment, the same degree of expectation may be anticipated for microorganisms isolated from a totally unrelated environment (Ojo, 2005).

The present study which aimed at investigating the possibility of using a mushroom species to degrade the oil in oil-polluted soil shows that *P. florida* has the potentials of doing so. The result clearly demonstrates that if suitably developed, application of a carrier-based indigenous mushroom can be used to remediate soil contaminated with crude oil. Organic carbon content of the polluted soil increased with increasing oil contamination but declined when the concentration became toxic to the fungus as shown in Table 1. The highest level of increase of carbon content was from 7.07% at 15% engine oil in the soil, followed by a low decrease from 6.70 to 6.48% at 20 and 25% engine oil in the soil, respectively. This result is similar to that reported by Adenipekun and Isikhuemhen (2008) where the highest level of carbon content was 7.27% at 10% engine oil in the soil and later decreased to 6.79% at 40% oil in the soil. Atlas and Bartha (1972) also reported that an addition of crude oil to the soil enriches micro-organisms capable of utilizing hydrocarbons. *P. florida* seemed to have improved the organic carbon content of the soil compared to the control after 60 days of incubation, through the breakdown of the applied engine oil. Odjegba and Sadiq (2002) reported that engine oil usually contains chemical additives, which include amines, phenols, benzene, calcium, zinc, barium, magnesium, phosphorus, sulphur and lead. It was also their opinion that metals present in spent lubricating oil are not necessarily the same as those present in unused lubricant.

The role of fungi in degradation of recalcitrant hydrocarbons has been possible due to their ability to grow under environmentally stressed conditions such as poor nutrient status. This biological agent provides an option in reducing environmental pollutants in the soil.

Table 1. Changes in organic carbon content of the soil contaminated with engine oil after 60 days of incubation of *P. florida* when compared to their control.

Treatment [Contamination with engine oil (%)]	% value of organic carbon after 60 days	
	Inoculated soil	Control
5	6.20	5.01
10	6.27	5.21
15	7.07	5.81
20	6.70	4.73
25	6.48	4.01

Each reading is a mean of four replicates.

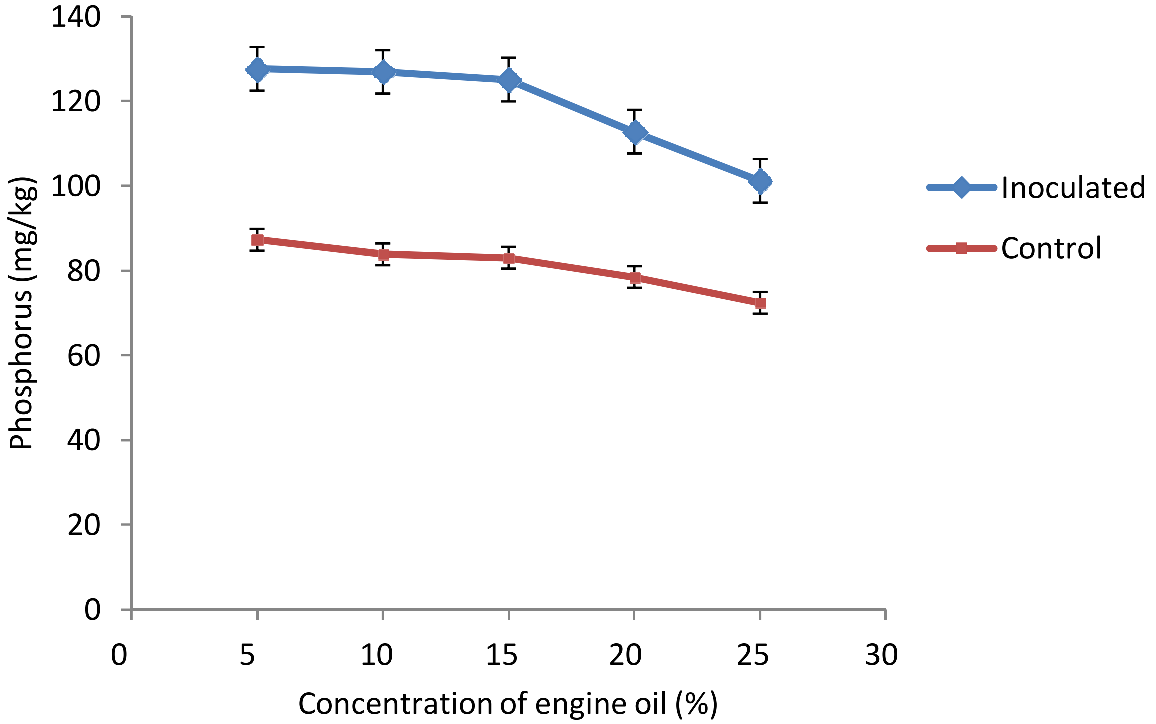

Figure 1. Changes in phosphorus content of soil contaminated with engine oil after 60 days of incubation of *P. florida*.

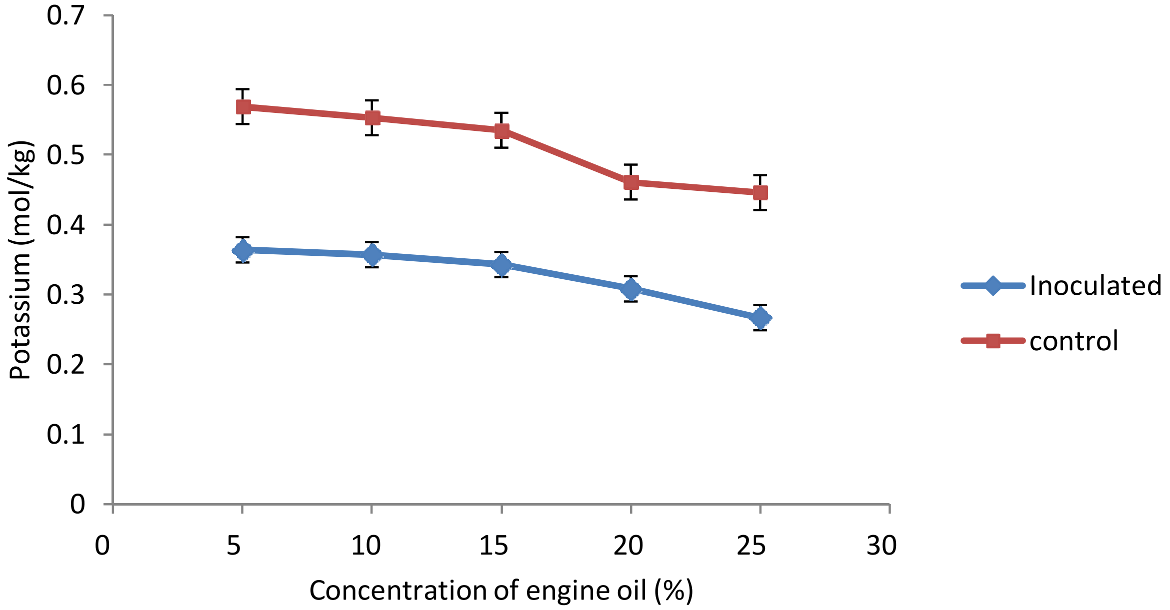

Figure 2. Changes in potassium content of the soil contaminated with engine oil after 60 days of incubation of *P. florida*.

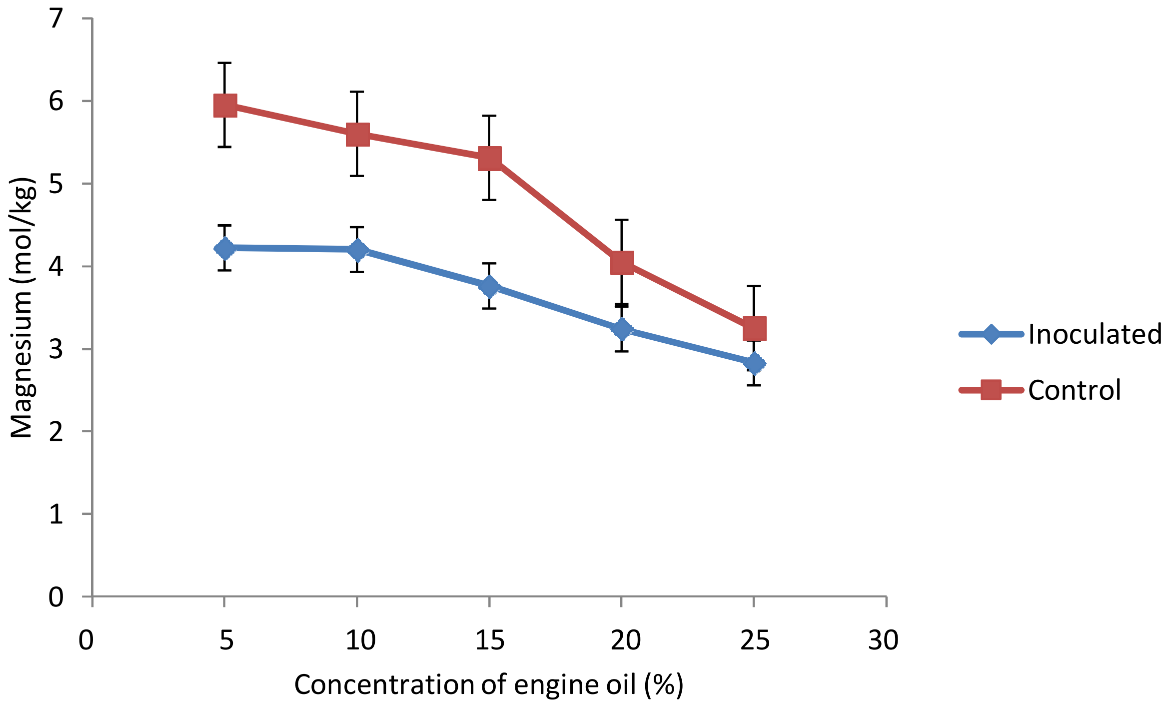

Figure 3. Changes in magnesium content of the soil contaminated with engine oil after 60 days of incubation of *P. florida*.

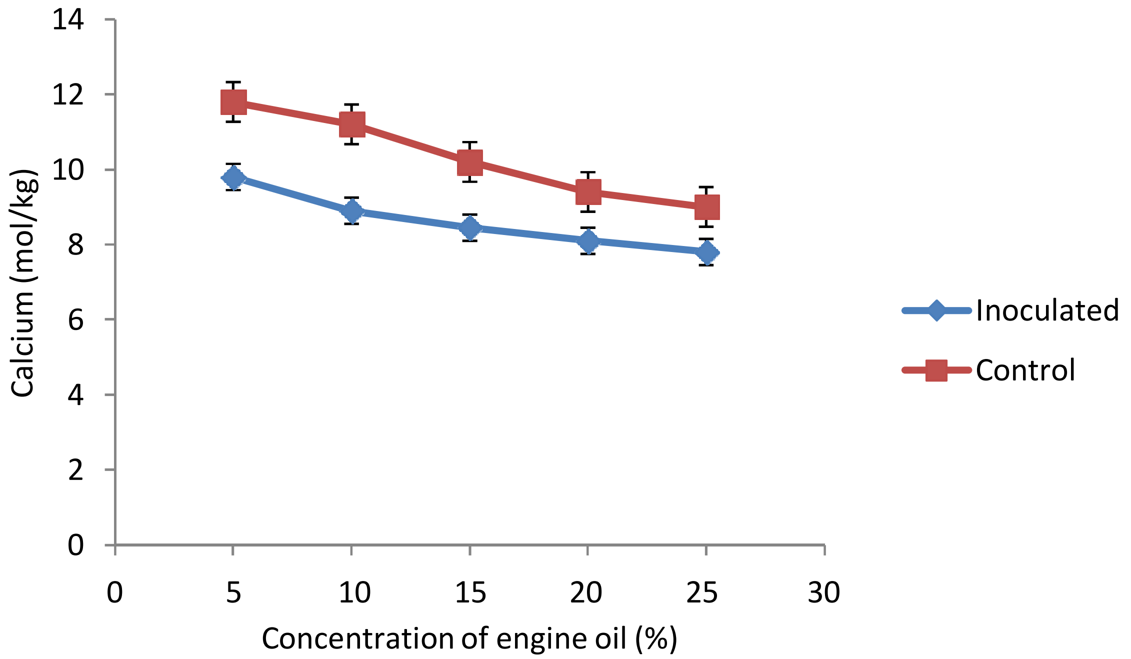

Figure 4. Changes in calcium content of the soil contaminated with engine oil after 60 days of incubation of *P. florida*.

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