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Full Length Research Paper

Bioremediation of engine-oil polluted soil by *Pleurotus tuber-regium* Singer, a Nigerian white-rot fungus

Adenipekun, Clementina Oyinkansola

Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. E-mail: oyinpek@yahoo.com. Tel: 08033683516, 08055265186. Fax: 234- 02-8103043.

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White-rot fungi have been used in various parts of the world for bioremediation of polluted sites. *Pleurotus tuber-regium* was noted to have the ability to increase nutrient contents in soils polluted with 1 - 40% engine-oil concentration after six months of incubation. *P. tuber-regium* increased organic matter, carbon and available potassium 5.19%, 2.99% and 0.97 meq/100 g respectively compared to 4.41%, 2.56% carbon and 0.66 meq/100 g, respectively in the control. However, higher values of 0.32% nitrogen, 11.42 ppm phosphorus and pH 6.94 were obtained in the control compared to 0.16% nitrogen, 9.32 ppm phosphorus and pH 5.93 in soils incubated with the fungus. The fungus brought about an increase in copper content in engine oil polluted soils at 10% concentration followed by a decrease at 20 and 40% concentrations. Bioaccumulation of zinc and nickel was recorded at 20% engine-oil concentrations.

Key words: Bioremediation, engine-oil polluted soil, nutrient contents, heavy metals, white-rot fungi.

INTRODUCTION

Bioremediation is the enhancement of live soil organisms such as fungi, bacteria and plant to break down hydrocarbon and organic contaminants. It involves the application of organisms and nutrients such as phosphate and nitrogen to the contaminated soil. Nitrate and phosphate supplements enhance biodegradation of oil (Atlas and Bartha, 1992). Bioremediation involves the transformation of complex or simple chemical compounds into non-hazardous forms by biological agents resulting in materials of a higher nutritive value or simply reducing the final bulk of the product (Grady, 1985). This then gives rise to a variety of products most of which will be much more water-soluble than the parent hydrocarbon.

White-rot fungi have been known for their ability to degrade lignin, a non repeating structural polymer found in woody plant and this ability enables them to degrade xenobiotic pollutants (Crawford, 1981, Bumpus Aust, 1987). *Pleurotus tuber-regium* (a white-rot fungus) has been reported to ameliorate crude oil polluted soil and the resulting soil sample supported germination and seedling of *Vigna unguiculata* (Isikhuemhen et al., 2003). Many studies have reported the use of *Pleurotus* species in bioremediation exercises (Baldrian et al., 2000). Adenipekun and Fasidi (2005) reported the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil. They found that nutrient contents were generating higher after 6 months of incubation except potassium levels, which were not significantly different from the control. Adedokun and Ataga (2007) also investigated the effects of sawdust and waste cotton as soil amendment and bioaugmentation with *Pleurotus pulmonarius* on soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (*V. unguiculata* L. Walp). There was a significant improvement (P = 0.05) on the growth of cowpea when polluted soil was amended and bioaugmented with *P. pulmonarius* after one month of incubation as compared with planting on polluted soil with no amendments and bioaugmentation. This work aimed at investigating the bioremediation of engine oil polluted soil by *P. tuber-regium*.

MATERIALS AND METHODS

Fungal cultivation and incubation

The cultivation conditions were according to the method of Baldrian et al. (2000) and modified as follows: 100 g of sterilized soil mois-

Table 1. Nutrient contents of engine-oil contaminated soils incubated with *P. tuber-regium* for 6 months.

Treatment (engine oil)	Organic matter (%)	Carbon (%)	Nitrogen (%)	Phosphorus (ppm/g)	Available Potassium (meq/100 g)	pH
Control	1.90[c]	1.10[c]	0.15[a]	7.61[a]	1.17[a]	5.99[abc]
1%	2.7[c]	1.61[a]	0.15[a]	7.67[a]	1.17[a]	6.00[abc]
2.5%	3.19[c]	1.85[c]	0.22[a]	8.38[b]	1.12[a]	6.31[a]
5%	9.42[a]	5.37[ab]	0.24[a]	10.65[a]	1.02[a]	5.59[c]
10%	9.47[a]	5.49[a]	0.14[a]	10.89[a]	0.97[a]	5.63[c]
20%	7.49[a]	4.34[b]	0.12[a]	10.40[a]	0.89[a]	5.92[abc]
40%	2.06[c]	1.19[c]	0.09[a]	9.65[a]	0.39[b]	6.07[ab]

Each value is the mean of 3 replicates.
Mean values in same column followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

tened with 75% distilled water (w/v) were weighed into 9 x 9 x 4 cm (350 cm^3) jam bottles and then mixed thoroughly with engine oil concentrations (1, 2.5, 5, 10, 20 and 40%). 20 g of clean rice straw was laid on the contaminated soil in each bottle, covered with aluminum foil and autoclaved at 15 lbs pressure for 15 min. Each bottle was then inoculated with two agar plugs of a vigorously growing mycelium of *P. tuber-regium* using a 7 mm sterile cork borer. The bottles were incubated at room temperature for 3 – 6 months.

Engine oil was not added to the control experiment. At 6 months after incubation, the mycelial-ramified waste was separated from the soils and analyzed for physio-chemical parameters after air-drying.

Soil pH, organic carbon, nitrogen, phosphorus and potassium

The soil pH was determined according to the procedure of Bates (1954). Organic matter, nitrogen, phosphorus and potassium constituents were determined according to the method of AOAC (2005).

Heavy metal contents

The soil was analyzed for heavy metal content by first ashing the soil and then analyzing by flame atomic absorption spectrophotometer (Crosby, 1977).

RESULTS AND DISCUSSION

Table 1 shows that in soils contaminated with engine oil and incubated with *P. tuber-regium* for 6 months, the organic matter, carbon and phosphorus increased up to 10% engine oil treatment and decreased, thereafter. Nitrogen increased up to 5% engine oil treatment and decreased, whereas available potassium decreased gradually from 1.17 meq/100 g to 0.30 meq/100 g in 40% engine-oil contaminated soil. The pH values did not show any definite trend except at 5 and 10% concentration engine oil where the values were not significantly different at $p>0.05$ from each other according to Duncan's multiple range test.

Figure 1 shows the copper content in mycelia of *P. tuber-regium* 6 months after contamination with engine oil. The copper content subsequently increased gradually to 10% recording 6.08 mg/g dry wt. followed by a decrease to 5.36 mg/g dry wt and 3.48 mg/g dry wt at 20 and 40% engine-oil concentration, respectively. Figure 2 shows that Zn and Ni values increased gradually to 20% engine oil contaminated soil followed by a decrease in both metal contents at 40% engine oil concentration.

The results show that the organic carbon in engine oil contaminated soil was higher than in uncontaminated soil. This is probably due to the effect of contamination with hydrocarbon in the soil. An increase in nutrient contents was also observed in soils treated with engine oil compared with the control. This is in line with the observation of Atlas and Bartha (1972) who reported that the addition of crude oil to an ecosystem will enrich primarily the micro-organisms capable of utilizing the hydrocarbons and secondary micro-organisms capable of utilizing metabolites produced by the oil-utilizing micro-organism. Lehtomake and Niemela (1975) reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contamination. This is contrary to the findings in this study, which showed higher values except at high concentrations of 20 and 40%. In this study, the pH range of 6.65 - 7.15 was observed in oil-contaminated soils with no fungal contamination. Verstrate et al. (1975) found optimal activity for microbial degradation at a pH of 7.4 and considerable inhibition at pH 4.5 and 8.5. The inoculation of *P. tuber-regium* on contaminated soil resulted in greater nutrient contents compared to the control. A greater soil nutrient distribution of 5.19% organic matter, 2.99% carbon and 0.9 7meq/100 g potassium was observed in soils contaminated with engine oil after six months of incubation compared to the control, which recorded 4.41% O.M., 2.56% C and 0.66 meq/100 g K. This indicates that biodegradation has taken place.

In this study, a reduction in nutrient contents of contaminated soils after introduction of the white-rot fungi at higher levels of 10 - 20% engine oil concentration was

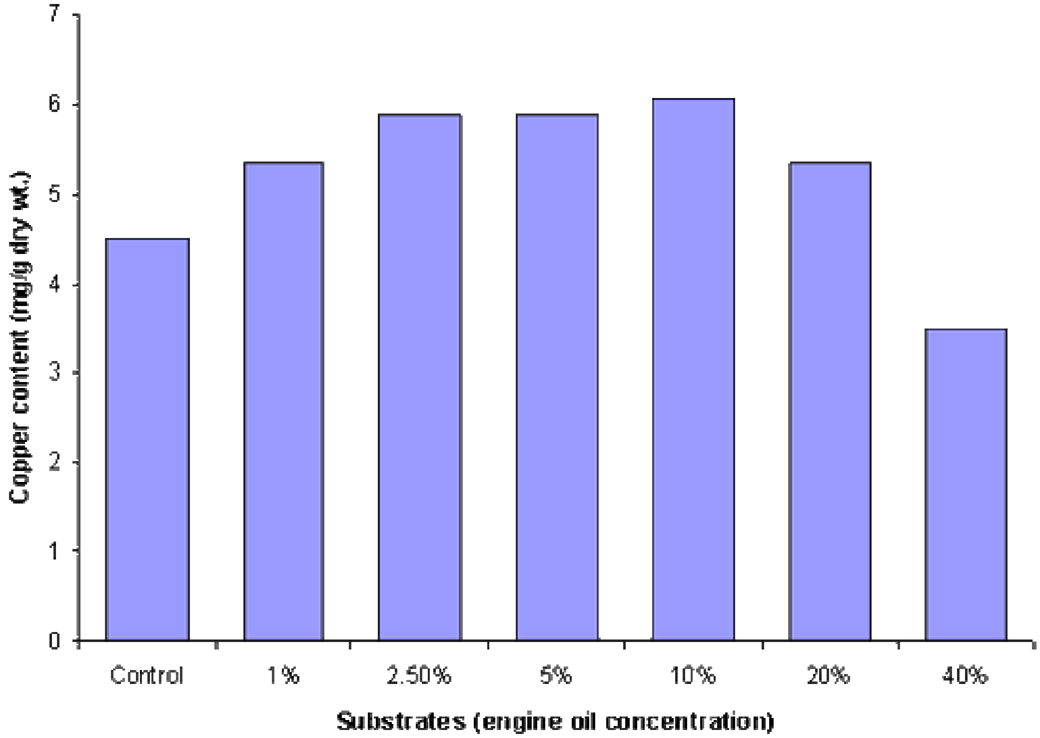

Figure 1. Copper content in *P. tuber-regium* mycelia 6 months after incubation on engine-oil contaminated soil.

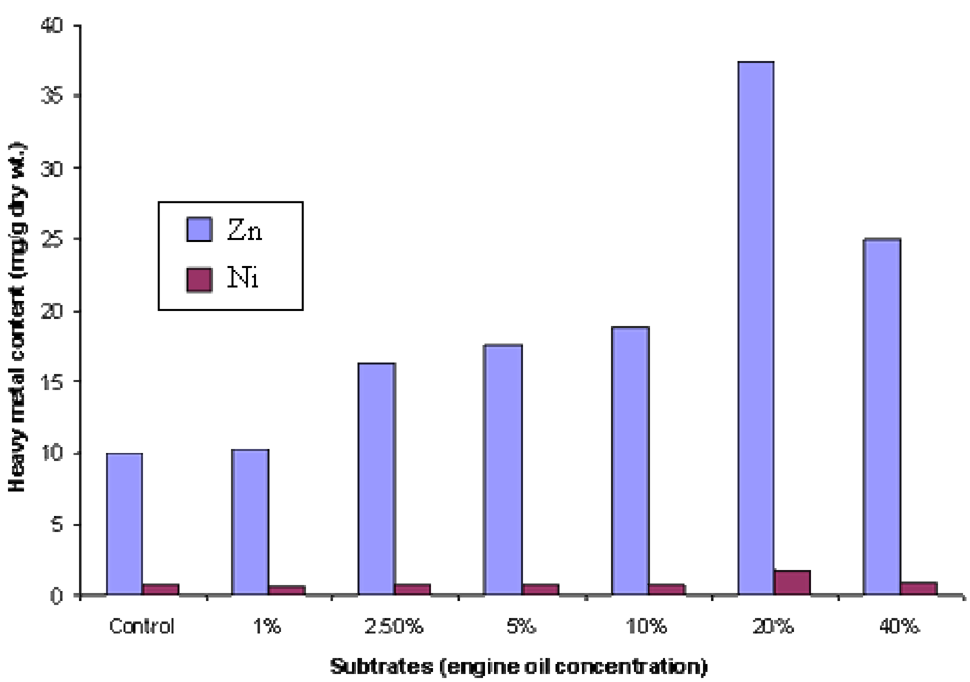

Figure 2. Heavy metal contents in *P. tuber-regium* mycelia 6 months after incubation in engine-oil contaminated soil.

observed compared to lower levels of contaminated oil. This is in line with the findings of Calder and Lader, (1976) who reported that toluene and phenol may stimulate growth at low concentration but show bacterio-cidal action at high concentration and solubility of the crude oil components.

The introduction of the mushroom species brought an increase in nutrient contents of soils and reduction in heavy metals after six months of incubation. Hence, the fungus can be employed in decontaminating environment polluted with engine oil. Further research is, however, advocated in investigating the efficiency of these methods on crude oil contaminated soils and their toxicity on indigenous micro-organisms.

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