Research Journal of Agriculture and Environmental Management. Vol. 2(11), pp. 354-357, November, 2013
Available online at http://www.apexjournal.org
ISSN 2315 - 8719© 2013 Apex Journal International

Full Length Research Paper

Mycoremediation of engine oil-polluted soil using the white rot fungus, *Pleurotus florida* (Mont.) Singer, an edible fungus

Okwujiako, I.A.*, Okwulehie, I.C. and Igbojionu, V.O.

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.

Accepted 14 October, 2013

The ability of a white rot macro-fungus, *Pleurotus florida*, to degrade engine oil in contaminated soil was investigated with a view to ascertaining its efficacy in reducing the toxicity of polluted ecosystem. Sterile soil samples (100g) contained in polypropylene bags (12cm diameter x 30cm 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 – 30°C for 60 days. Soil samples from the polluted soil were analyzed after the incubation period for the total percentage of petroleum hydrocarbons lost and the effect of pH in the biodegradation. The total petroleum hydrocarbon (TPH) lost was highest at 5% concentration with 89.12% loss. Loss in TPH decreased sequentially to 65.92% at 25% concentration. The highest decrease was from 6.1 to 5.0 at 5% engine oil in the soil, followed ultimately by a slight decrease from 6.8 to 6.7 at 25% contamination, when compared to their controls. Statistical analysis revealed significant differences between the inoculated samples and their controls, indicating degradation of the polluting oil. The results are discussed against the background of hydrocarbon pollution in the natural environment and the potential of using *P. florida* in the intervention.

Key words: Mycoremediation, engine oil, *Pleurotus florida,* hydrocarbons, pollution.

INTRODUCTION

Nigeria is a major producer of crude oil in the world and pollution of the environment due to increased in oil spillage. Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds, which possess a measurable toxicity towards living systems (Nelson-Smith, 1973). The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment. Oil spillages into the environment have become a major problem. Spillages of used motor oils such as diesels or jet fuels and engine oil contaminate our natural environment with hydrocarbons. The hydrocarbons spread horizontally on the ground-water surface and partition into ground-water, soil pore spaces and to the surface of soil particles (Plohl *et al*., 2002). Hydrocarbon contamination of the air, soil and fresh water especially by polycyclic aromatic hydrocarbons (PAHs) has drawn public concerns because many are toxic, mutagenic, and carcinogenic (Cerniglia and Sutherland, 2001).

Recently, fungi have received considerable attention for their bioremediation potential that is attributed to the enzymes they produce that are involved in lignin breakdown and which degrade a wide range of recalcitrant pollutants, such as polyaromatic hydrocarbons, chlorophenols and pesticides (Bumpus *et al*., 1985). In addition, fungi have advantages over bacteria in that fungal hyphae can penetrate contaminated soil to reach the PAHs that have spread beyond the top layer of the soil. Engine oil, which is one of the major products of crude oil, constitutes a major source of pollution in our

*Corresponding author. Email: phylyke@yahoo.com

environment. With total dependence on engine oil by virtually all automobile machines, electric generating plants and other types of engines, greater quantities are being used than previously and the spent one usually regarded as condemned engine oil is discarded on the environment carelessly.

One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fibre (Stamets 1999b). These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. Wood-degrading fungi are particularly effective in breaking down pollutants (toxic components of substances including petroleum), as well as chlorinated compounds and certain persistent pesticides (Battelle, 2000).

The effect of oil on microbial populations depends upon the chemical composition of the oil and on the species of micro-organisms present. However, some crude oils contain volatile bacteriostatic compounds that must be degraded before microbial populations can grow (Atlas and Bartha, 1972). The focus of this paper is to evaluate the efficacy of the white rot fungus, *P. florida,* in the remediation of engine oil-polluted soil and the implication of pH in the degradation.

MATERIALS AND METHODS

The fungus *P. florida* used for this study was obtained from Dr. I.A. Okwujiako, which was cultured and 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. Rice straw used was collected from a rice farm in Bende Local Government Area of Abia State.

Spawn production

Spawn was prepared by the method described by Senyah *et al*. (1989). White guinea corn grains were thoroughly washed with tap water and soaked overnight. They were dispensed into spawn bottles and autoclaved at 121^{o}C for 1hour each day for 3 consecutive days. On cooling, the grains were inoculated with four 9mm mycelial discs each bottle,taken from a 14-day-old agar culture of *P. florida* and incubated at 28±2^{0}C for 14 days in darkness.

Fungal cultivation

A modified method of Baldrian *et al* (2000) was employed. One hundred gram aliquots of sterilized soil were weighed into polypropylene bags (12cm diameter x 30cm high) before sterilization. Different concentrations (5, 10, 15, 20 and 25% v/w) of engine oil were added and mixed thoroughly. Thirty grams of pieces of sterilized rice straw were laid on the contaminated soil. Each bag was inoculated with 7g 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. Data collected were analysed 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

After 60 days of incubation of the soil with *P. florida*, there was decrease in pH in all the different levels of the contaminated soil as shown in Figure 1. The highest decrease was from 6.1 to 5.0 at 5% engine oil in the soil, followed ultimately by a slight decrease from 6.8 to 6.7 at 25% contamination when compared to their controls. Adenipekun and Isikhuemhen (2008) reported a decrease in pH at 1% and 10% concentration of engine oil in the soil. According to Okwulehie *et al*., (2006), acidic pH supported the mycelial growth of *Pleurotus pulmonarius* at the pH range of 5.0 and 6.0. Similar studies also show that fungi are more tolerant to acidic medium than basic medium (Bilgrami and Verma, 1992). Hofrichter *et al*. (1999) revealed that white rot fungi are known to secrete organic acids into their substrate which presumably can lower the pH to levels optimum for their enzymes to function best. Dibble and Bartha (1979) reported that pH range of 5.0 to 7.8 favoured the degradation of oily sludge in the soil. The changes according to them were more at 0 to 20% crude oil in the soil.

Table 1 shows the percentage loss in total petroleum hydrocarbon (TPH) after 60days of incubation with *P. florida*. There was considerable loss in the total percentage of engine oil for all oil contaminated soils. The greatest loss was at 5% engine oil contamination with 89.12% loss and the lowest at 25% engine oil contamination with 65.92% loss. This is similar to the study done by Adenipekun and Isikhuemhen (2008) with *Lentinus squarrosulus* where the percentage of engine oil lost was highest at 1% concentration of engine oil contaminated soil recording 94.46% but decreased to 69.05% at 40% concentration of engine oil contaminated soil after 90 days of incubation. According to Stamets (1999b), mycelial mats are used for bioremediation because mycelia produce extra-cellular enzymes and acids that breakdown recalcitrant molecules such as: lignin and cellulose. Also lignin peroxidases dismantle the long chains of hydrogen and carbon making them

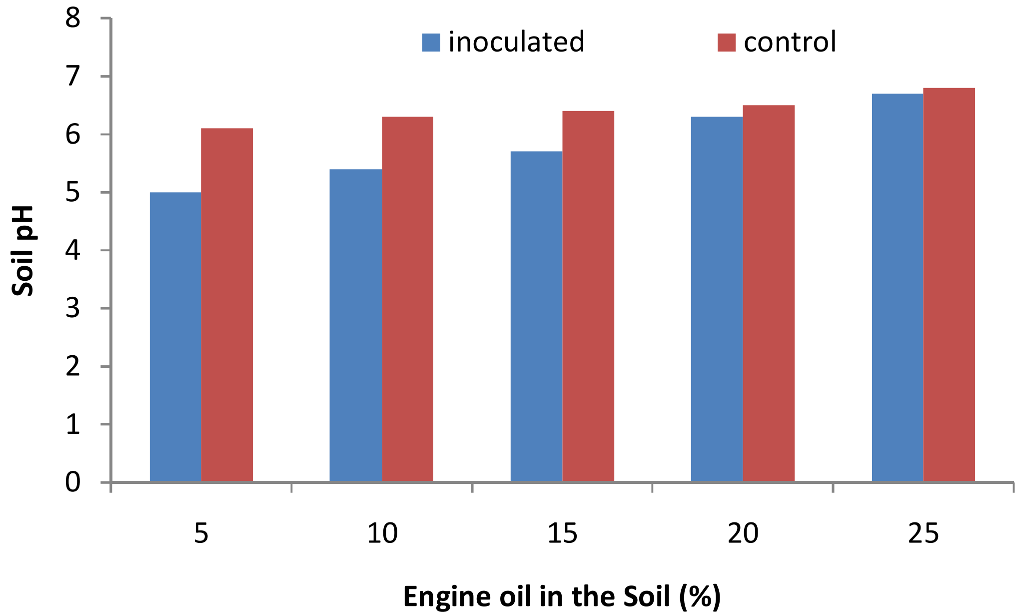

Figure 1. Effect of pH on degradation of engine oil polluted soil after 60days of incubation with *P. florida.*

Table 1. Total Petroleum Hydrocarbon (TPH) loss after 60 days of incubation of the soil with *P.florida TPH (mgkg^{-1})*

Treatment (Conc.of Engine oil)	Control(60days)	Inoculated(60days)	TPH (% loss)
5%	7507.1 ± 0.10	816.65±0.35	89.12
10%	8785.06 ± 0.96	1165.61±0.40	86.73
15%	9276.3 ± 0.10	1450.56±0.03	84.36
20%	10602 ± 0.84	2638.17±0.18	75.12
25%	11652.46 ±0.07	3970.78±0.22	65.92

Each reading is a mean of four (4) replicates ± standard error

effective at breaking apart hydrocarbon-the base structure common to oils, petroleum products and many other hydrocarbon pollutants.

Similarly, Adedokun and Ataga (2006) showed that there was growth of the mycelia of *Pleurotus tuber-regium, P. pulmonarius* and *Lentinus squarrosulus* on the engine oil-polluted potato dextrose agar (PDA) used for their work. It has been reported that spent sawdust cultures of *Lentinus edodes* removed 44-61% of the pentachlorophenol in sterile contaminated soil after 21days of incubation (Okeke *et al*., 1993). Rosado and Pitchel (2004) reported that a total of 67% oil was removed in sunflower/ mustard treated soil. Isikhuehmen *et al*., (2003) reported that white rot fungi bioremediated crude oil-polluted soil and resulted in improved percentage germination in *Vigna unguiculata*.

The use of fungi in bioremediation is of great importance as it can tolerate oil pollutants and equally grow on them to bring about breakdown of the toxic materials in an environmentally friendly manner.

REFERENCES

Adedokun, O.M., Ataga, A.E. (2006). Effects of crude oil and oil products on the growth of some edible mushrooms. *J. Appl. Sci. Environ. Manage.*, 10 (2): 91-93.

Adenipekun, O.C., Isikhuemhen, S.O. (2008). Bioremediation of engine oil polluted soil by the tropical white rot fungus, *Lentinus squarrosulus* Mont. (Singer). *Pak. J. Bio. Sci.* 11(12): 1634-1637.

Atlas, R.M. Bartha, R. (1972). Degradation and mineralization of petroleum by two bacteria isolated from coastal water. *Biotechnol.* Bioeng. 14: 297-308.

Baldrian, P., Inde Wiesche C., Gabriel, S., Nerud, F., Zadrazil, F. (2000). Influence of Cadmium and Mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil. *Applied Environ. Microbiol.* 66: 2471-2478.

Battelle, G.W. (2000). Putting Technology to Work: The very Essence of Technology Transfer and Commercialization. http://www.battelle.org./Environment/publications.

Bilgrami, K.S., Verma, R.N. (1992). *Physiology of fungi.* Vilkas Publishing House Hvt Ltd. London. P334

Bumpus, J.A., Aust, S.D., Wright, D., Tien, M. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. Science, 228: 1434-1436

Cerniglia, C.E., Sutherland, J.B. (2001). Bioremediation of polycylic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. In: Gadd, G.M. (ed) *Fungi in Bioremediation.* Cambridge Univ. Press, Cambridge. pp136-187.

Dibble JJ, Bartha R (1979). Effect of environmental parameters on the biodegradation of sludge. Applied Environ. Microbiol., 37: 729-739.

Hofrichter, M., Vare, T., Kalsi, M., Glakin, S., Scheibner, K, Fritsche, W., Hatakka, A. (1999). Production of manganese peroxidase and organic acids and mineralization of 14C-labelled lignin (14C – DHP) during solid state *fermentation* of wheat straw with white rot fungus Nematoloma frowardii. *Applied Enviorn. Microbiol.*, 65: 1864-1870.

Isikhuemhen, O.S., Anoliefo, G., Oghale, O. (2003). Bioremediation of crude oil polluted soil by the white rot-fungus, *Pleurotus tuberregium*(Fr). Sing. Environ. Sci. pollut. Res. 10: 108-112.

Nelson-Smith A (1973). *Oil Pollution and Marine Ecology.* New York, Plenum Press.

Okeke, B.C., Smith, J.E., Paterson, A., Waster-Crack, I.A. (1993). Aerobic metabolism of pentachlorophenol by spent sawdust culture of shiitake mushroom (*Lentinus edodes*) in soil. *Biotech. Lett.,* 15: 1077-1080.

Okwulehie, I.C., Okwujiako, I.A., Igbojionu, V. (2006). Studies on nutritional requirements and growth of *Pleurotus pulmonarius* (Fries) Quelet, an exotic mushroom . *Niger. J. Bot.* 19(2): 308-316.

Plohl, K., Leskovsek, H., Bricelji, M. (2002). Biological degradation of motor oil in water. *Acta. Chim. Slov.* 49: 279-289.

Rosado, E.D., Pitchel, J. (2004). Phytoremediation of soil contaminated with used motor oil II greenhouse studies. *Environ. Eng-Sci.,* 21: 169-180.

Senyah, J.K., Robinson, R.K., Smith, J.F. (1989). The cultivation of the oyster mushroom *Pleurotus ostreatus* (Jacq. Ex.Fr) Kummer on cocoa shell waste. Mush. Sci., XII (part II), 207-17.

Stamets, P. (1999b). Helping the ecosystem through mushroom cultivation. *Fungi Perfecti. Mushrooms and the Ecosystem.* http://www.fungi.com/mycotech/mycova html.