Management of Polluted Soils by a White-Rot Fungus: *Pleurotus pulmonarius*

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Abstract

There has been increasing interest in the application of organisms and nutrients to contaminated soils for effective degradation of oil. The white-rot fungus Pleurotus pulmonarius was investigated for its ability to mycoremediate polyaromatic hydrocarbons (PAH) and heavy metal content in cement and battery wastes polluted soils. The effect of the incubation periods on the contents of cement and battery polluted soil incubated with P. pulmonarius after six and ten weeks was determined.

A general increase in the carbon, organic matter, phosphorus and potassium was observed while a decrease in percentage nitrogen, calcium and pH was observed after 6 and 10 weeks. The heavy metal content of the two polluted soil showed that lead (8 ppm) was constant at 6 and 10 weeks while significant decrease in copper, manganese and nickel was observed in cement contaminated soil. However there was a gradual decrease in lead content of batter polluted soil while copper was not utilized at all. The polyaromatic hydrocarbons (PAH) also decreased from 6.86 in control to 0.56 after 10 weeks of incubation. All of these results show that the cement polluted soil was effectively remediated by the white-rot fungus.

Keywords: *Pleurotus pulmonarius, hydrocarbons, cement polluted soil, mycoremediation.*

Introduction

Bioremediation is a biotechnological approach of rehabilitating areas degraded by pollutants or otherwise damaged through mismanagement of ecosystem. It is the ability of micro-organisms to degrade, transform, or detoxify organic compound polluted areas by converting undesirable and harmful substances into non-toxic compound (Integra Environmental 2011). Bioremediation of hydrocarbon contaminated soil by the use of micro-organisms has been established to be efficient, inexpensive, adaptable, and to be an environmentally sound treatment method. Several factors contribute to or affect the rate of degradation of hydrocarbons in a soil and they include temperature, physical and chemical nature of the pollutant, composition of the hydrocarbons, bioavailability of the substrates and microbial population and types (Margesin *et al*. 2000). Examples of pollutants or hazardous compounds include, polycyclic aromatic hydrocarbon (PAH), pentachloro-phenol's (PCP), polychlorinated biphenyls (PCB), benzenes, toluene, ethyl benzene, etc., but this work will be centered on PAH in cements and polluted soil. The above mentioned compound is persistent in the environment and is known to have carcinogenic or mutagenic effects.

White-rot fungi such as *Phanerochaete Chrysosporium, Pleurotus ostreatus, P.tubes-regium, P. Pulmonarius* and *Lentinus Squarrosuhus*, etc., have been used in the bioremediation of polluted soil and bioaccumulation of heavy metals (Adenipekun and Omoruyi 2008). This white-rot fungi *P. pulmonarius*, is wood decaying basidomycetes which is capable of degrading not only lignin but also variable recalcitrant environmental pollutants due to its ability to secrete lignolytic enzymes such as lignin peroxidiase, manganese peroxidase and laccases which aid the degradation process (Ogbo *et al*. 2006). The objective of this study is to evaluate the ability of *Pluerotus pulmonarius* in remediating soil polluted with cement and battery, and the ability of the fungi to degrade lignin in the rice strew used as the lignocellulose substrate.

Materials and Methods

The soil samples used for this experiment were collected from West African Portland cements dump site in Sagamu, Ogun State and a battery dump site in Ogunpa area of Ibadan, Oyo state. The main substrate (rice straw) which is lingocellulolytic in nature was collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The substrate was cut into 3-5 cm long with a guillotine and then soaked in water for 30 minutes to leach herbicides out of rice straw, the straw was squeeze through a muslin cloth until no more water oozed out.

Fungi Cultivation and Incubation

Forty grams of rice straw were laid on 200g contaminated soil in a jam bottle 350 cm^3 covered with aluminum foil and autoclaved at 121°C for 15 minutes according to the modified method of Adenipekun and Fasidi (2005). Each bottle was inoculated with 10g of vigorously growing spawn of the fungi and then incubated at room temperature for specific period of time as stated. *P. pulmonarius* was grown on rice straw laid on cement contaminated soil and incubated for 2 months. This was also done for battery contaminated soil. Two sets of controls were set up and the experimental set-up was replicated two times. After incubation, the mycelia ramified substrate was carefully separated from the soil layer to ensure that the soil particle did not mix with it. All soil samples were analyzed for physiological parameters after air-drying.

Nutrient Content Analysis

The soil pH was determined by weighing 20g of air dried soil samples into soil beaker 200 ml of distilled water was then added, after which mixture was stirred manually for 5 minutes with a glass rod and allowed to stand for 30 minutes. The pH was then measured using a pH meter (Bates 1954). Organic carbon, percentage nitrogen, phosphorous and potassium content were determined according to the method of the Association of Official Analytical Chemists (AOAC 1980).

Total Petroleum Hydrocarbon (TPH) and Heavy Metal Analysis

Total petroleum hydrocarbon content of the soil was determined using a Fourier transform infrared spectrometry. The heavy metals, such as Pb, Cu, Zn, Ni, Cd, Fe, and Mn, in the soil were determined using aromatic absorption spectroscopy after double acid extraction. This technique does not make use of completely digested soil samples.

Determination of Lignin Content of Rice Straw

The acid detergent fraction (ADF) method was use to determine the lignin content in the rice straw and this is done according to the method of Van Soest and Wine (1968).

Statistical Analysis

The obtained data were analyzed using ANalysis Of VAriance (ANOVA) and the means were separated by Duncan’s Multiple Range Test (DMRT) developed by Duncan (1955).

Results

The result of nutrient contents in battery polluted soils incubated with *Pleurotus pulmonarius* is shown in Table 1. After 10 weeks of incubation, the organic carbon content of the soil was increased from 0.39% in control to 1.33%, the organic matter from 0.67% to 2.29%, phosphorus from 63.6 ppm/g to 110.62 ppm/g and potassium from 0.57 meq/100g to 1.6 meq/100g.

Table 1. Nutrient content in battery polluted soils incubated with *Pleurotus pulmonarius*.

Incubation period	Carbon (%)	Organic matter	Nitrogen (%)	Phosphorus	Calcium (meq/100g)	Potassium (meq/100g)	pH
Control	0.39[c]	0.67[c]	0.25[a]	63.6[a]	27.68[a]	0.57[b]	5.90[a]
6 weeks	1.01[b]	1.75[b]	0.10[b]	109.08[b]	11.20[b]	0.46[c]	5.56[b]
10 weeks	1.33[a]	2.29[a]	0.02[b]	110.62[b]	10.07[b]	1.60[a]	4.02[b]

However, the nitrogen content was reduced from 0.25 to 0.02% after 10 weeks, the calcium content of the soil also decreased from 27.68 meq/100g to 10.07 meq/100g. Similarly, the pH of the soil sample decreased from 5.90 to 4.02. The parameters were found to be significantly different from the control both at 6 and at 10 weeks of incubation.

Table 2 shows heavy metal content in battery polluted soil incubated with P. *pulmonarius*. After incubation for 10 weeks, there was reduction in the heavy metal content of the soil, Pb from 256 to 204, Mn from 32 to 14.63 and Ni from 0.50 to 0.05, but Cu remained the same even after 10 weeks of incubation, thus indicating that there was no significant difference in Cu, but there was significant difference in Pb, Mn, and Ni.

Table 2. Heavy metals in battery polluted soils incubated with *Pleurotus pulmonarius*.

Incubation period	Lead (Pb)	Copper (Cu)	Manganese (Mn)	Nickel (Ni)
Control	256[a]	0.005[a]	32.0[a]	0.50[a]
6 weeks	226[b]	0.005[a]	20.0[b]	0.05[b]
10 weeks	204[c]	0.005[a]	14.63[c]	0.005[c]

Table 3. Nutrient content in cement soil incubated with *Pleurotus pulmonarius*.

Incubation period	Carbon (%)	Organic matter (%)	Nitrogen (%)	Phosphorus (pm/g)	Calcium (meq/100g)	Potassium (meq/100g)	pH
Control	0.78[c]	1.34[c]	0.26[a]	36.9[c]	66.32[a]	0.45[c]	7.55[a]
6 weeks	1.48[b]	2.56[b]	0.01[b]	210.6[b]	59.36[b]	0.65[b]	7.54[b]
10 weeks	1.51[a]	2.84[a]	0.01[b]	268.4[a]	40.1[c]	1.61[a]	6.69[c]

Table 3 shows the nutrient content in cement battery polluted soil incubated with *Pleurotus pulmonarius*. After incubation for 10 weeks, there was increase in the organic carbon, organic matter, phosphorus, and potassium content in the cement contaminated soil from 0.78% to 1.51%, 1.34% to 2.84%, 36.0 ppm/g to 268.4 ppm/g, and 0.45 meq/100g to 1.6 meq/100g, respectively. The nitrogen, calcium content, and pH of the cement contaminated soil decreased from 0.26 to 0.01%, 66.32 meq/100g to 40.1 meq/100g, and 7.55 to 6.9, respectively, after 10 weeks of incubation.

Table 4 shows heavy metal content in cement polluted soil incubated with *P. pulmonarius*. Incubation after 10 weeks showed a reduction in the heavy metal content of the soil: Cu from 2.6 to 0.005, Mn from 2 to 0.003, Nickel from 0.04 to 0.05, while lead Pb remained the same after 10 weeks of incubation.

Table 4. Heavy metal in cement polluted soil incubated with *Pleurotus pulmonarius*.

Incubation period	Lead (Pb)	Copper (Cu)	Manga-nese (Mn)	Nickel (Ni)
Control	8.0[a]	2.6[a]	2.0[a]	0.40[a]
6 weeks	8.0[a]	0.005[b]	0.03[b]	0.05[b]
10 weeks	8.0[a]	0.005[b]	0.03[b]	0.05[b]

Table 5 shows the effect of incubation period on the polycyclic aromatic hydrocarbon (PAH) of battery and cement polluted soil incubated with *P. pulmonarius.* As the incubation period increased, the PAH in battery polluted soil increased from 0.125 (in control) to 0.565 after 6 weeks, and then to 1.8 after 10 weeks, while the PAH in cement polluted soil reduced from 6.86 (in control) to 1.8 after 6 weeks, and then to 0.56 after 10 weeks of incubation.

Table 5. Effect of incubation period on the Polycyclic Aromatic Hydrocarbon (PAH) of battery and cement polluted soil inoculated with *Pleurotus pulmoarius*.

Incubation period	Polycyclic Aromatic Hydrocarbon in Battery polluted soil	Polycyclic Aromatic Hydrocarbon in Cement polluted soil
Control	0.125[b]	6.86[a]
6 weeks	0.565[b]	1.80[b]
10 weeks	1.8[a]	0.56[c]

Table 6 shows the effect of soil type on loss of lignin, ash and organic matter content of rice straw incubated with *P. pulmonarius*. The percentage of lignin, ash and organic matter was higher in rice straw on battery polluted soil with values 6.04, 8.39, and 71.14, respectively, than that rice straw on cement polluted soil with values 4.01, 7.80, and 62.32, respectively, thus indicating that the loss of lignin, ash and organic matter in battery polluted soil is lesser compared to the lost experienced in rice straw on cement polluted soil based on soil type.

Note: In Tables 1-6, each value is the mean of 4 replicates. Mean values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Table 6. Effect of *P. pulmonarius* on lignin, ash and organic matter content of rice straw.

Soil type	% Lignin	% Ash	% Organic Matter
Control	11.24[a]	15.76[a]	84.24[a]
Rice straw on Cement-polluted soil	4.01[c]	7.80[c]	62.32[c]
Rice straw on Battery-polluted soil	6.04[b]	8.39[b]	71.14[b]

Discussion

The results obtained from this study showed an increase in the nutrient content of the soil with the increase of the incubation period. Increase in percentage organic carbon and organic matter in soil incubated with *P. pulmonarius* was also observed with the increase of the incubation period, thus indicating that a degradation must have taken place. This is similar to the work done by Adenipekun and Omoruyi (2008) in bioremediation of cement and battery polluted soil, where higher organic carbon and organic matter were recorded compared to control samples resulting in remediation of the samples.

An increase in phosphorus and potassium also occurred in both battery and cement polluted soil from 63.6 ppm/g to 110.6 ppm/g, 0.57 ppm/g to 1.60 ppm/g, and from 36.9 meq/100g to 286.4 meq/100g, 0.45 meq/100g to 1.6 meq/100g, respectively. This indicates that a degradation has occurred. The reduction in nitrogen and calcium observed in both soil samples showed that *P. pulmonarius* accumulated the calcium from both soil samples. The reduction is higher in cement contaminated soil due to the fact that cement is rich in calcium.

The reduction in the pH of the soil after the inoculation or introduction of *P. pulmonarius* from 7.55 to 6.69 after 10 weeks for cement polluted soil showed that microbial activities actually occurred in the soil. This is similar to the findings of Adenipekun and Omoruyi (2008) where they observed a decrease from 7.55 to 7.11 for cement polluted soil, and from 5.90 to 5.62 after 2 months of incubation. This is also in agreement with the report of Benneth *et al*. (2002).

This study also showed the reduction in the heavy metal content of both polluted soil samples, which indicates that *P. pulmonarius* has accumulated the heavy metals present in the soil. But for Lead (Pb) and Copper (Cu), which remain the same even after incubation for 10 weeks in cement and battery polluted soil, respectively, *P. pulmonarius* had no effect on them.

Furthermore, this study indicated that *P. pulmonarius* degraded rice straw and reduced the lignin content of the soil samples from 11.24% to 4.01% for cement polluted soil, 11.24 to 6.04% for battery polluted soil sample, while the ash content of cement and battery polluted soil reduced from 15.76% to 7.80% and 15.76% to 8.39%, respectively. This is similar to the findings of Adenipekun and Omoruyi (2008) and also the findings of Zadražil (1977).

P. pulmonarius has proved its ability to enhance the nutrient content of polluted soils, thus remediating the soils.

References

Adenipekun, C.O.; and Fasidi, I.O. 2005. Bioremediation of oil polluted soil by *Lentinus subnudus*, a Nigerian white rot fungus. African Journal of Biotechnology 4(8): 796-8, August.

Adenipekun, C.O.; and Omoruyi, O.M. 2008. Bioremediation of polluted soil by *Pleurotus ostreatus*. Nigerian Journal of Botany 21(2): 274-9.

AOAC. 1980. Official methods of analysis. 13th ed., Association of Official Analytical Chemists (AOAC), Washington, DC, USA.

Bates, R.G. 1954. Electrometric pH determinations: Theory and practice. John Wiley and Sons, Inc., New York, NY, USA.

Bennet, J.W.; Wunch, K.G.; and Faison, B.D. 2002. Use of fungi in biodegradation. *In*: C.J. Hurst (ed.) Manual of environmental microbiology. ASM Press, Washington, DC, USA, pp. 960-71.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Integra Environmental. 2011. Bioremediation overview. Integra Environmental, Houston, TX, USA. Available: <http://www.integraenvironmental.com/bioreovernew.htm>.

Margesin, R.; Walder, G.; and Schinner, F. 2000. The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil. Acta Biotechnologica 20(3-4): 313-33.

Ogbo, M.E.; Okhuoya, J.A.; and Anaziah, O.C. 2006. Effect of different levels of spent lubricating oil on the growth of *Pleurotus tuber-regium* fries singer. Nigerian Journal of Botany 19(2): 266-70.

Van Soest, P.J; and Wine, R.H. 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. Journal of the Association of Official Analytical Chemists 51: 780-5.

Zadražil, F. 1977. The conversion of straw into feed by *Basidiomycetes*. Applied Microbiology and Biotechnology 4(4): 273-81, December.