MYCOREMEDIATION OF SPENT AND FRESH CUTTING FLUIDS POLLUTED SOIL WITH A WHITE ROT FUNGUS Pleurotus ostreatus (Jacq.) Fr. P. Kumm.

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ABSTRACT

The challenge of overcoming detrimental effects of soil contamination has attracted increasing attention globally because of its carcinogenic, mutagenic and toxic effects on the ecosystem. Remediation is therefore required for such contaminated soil before its application for beneficial purposes such as crop cultivation. In this pilot study, Pleurotus ostreatus was employed to mycoremediate cutting fluids contaminated soil. Soil was contaminated with 10 %, 20 % and 30 % of spent and fresh cutting fluids and analysed in triplicates for some physiochemical properties before and after incubation with P. ostreatus for 2 months to assess its mycoremediating capability. The properties include organic carbon, organic matter, total nitrogen, phosphorus, potassium, pH, heavy metals, Cation Exchange Capacity (CEC) and Total Petroleum Hydrocarbon (TPH) contents. The general increment in the concentrations of organic carbon, organic matter, total nitrogen, phosphorus and slight reduction in heavy metal contents were indicative of degradation of organic and inorganic contaminants. There was no significant difference in CEC of the spent cutting fluid contaminated soil after incubation with P. ostreatus except at 30 % contamination level. However, significant difference in CEC of the fresh cutting fluid contaminated soil at 10 %, 20 %, 30 % contamination levels was observed after incubation with the fungus. TPH loss was observed during incubation with the fungus, ranging from 16.2% to 36.8% for spent cutting fluid and 15.2 % to 32.1 % for fresh cutting fluid contaminated soil samples. This loss in petroleum hydrocarbon content indicates biodegradation of organic contaminants in the soil.

Key words: Mycoremediation, cutting fluids, Pleurotus ostreatus, nutrients contents.

INTRODUCTION

Cutting fluids as a lubricants, is a complex mixture of hydrocarbons, fatty acids, emulsifiers, organo-corrosion inhibitors, amines and glycols that may also contains organo-boron complexes, copolymer, esters, alcohol, halogenated hydrocarbon, organophosphate and
phosphate (Gannon et al., 1981; Ejoh et al., 2012). They are applied in metal works such as cutting, grinding, drilling, forming and tapping. Waste metal working fluids as coolants and liquid lubricants are not to be dispose of indiscriminately, due to their toxicity in biosphere and high oxygen chemical demand in hydrosphere. The toxicity is largely due to biocides added into the blend to prevent bacteriological attack during its lifetime (Gérald, 2004). These toxic chemicals in the terrestrial environment clearly pose a significant risk to the quality of soils, plants, natural waters and human health (USEPA, 2003).

Environmental pollution with petroleum and petrochemical products has gained much attention globally in recent times due to increase in lubricants usage by different types of automobiles and machineries (Husaini et al., 2008). Different technologies used to remediate hydrocarbon contaminated soils are biological, chemical and physical technologies such as incineration, use of landfills, solidification and biological treatments (Scragg, 2005; Adams et al., 2009). Among these technologies, biological technology (mycoremediation) is of great interest by offering an eco-friendly and low cost bioremediation technique because it is a natural process which does not produce toxic bye products and provides a permanent solution as a result of complete mineralization of the contaminants in the environment (Perelo, 2010).

Fungi have been harnessed by humans for several applications for thousands of years. They are well known to degrade, or cause deterioration of a wide variety of materials and compounds (Sing, 2006). Among fungi, white rot fungi has shown a great potential in the degradation of a wide range of organo-persistent pollutants with the production of extracellular peroxidases (manganese and lignin peroxidase and laccases) that catalyze the breaking down of organic compounds (Gadd, 2001). Kalac and Svoboda (2004) reported that mushrooms are capable to build up large concentrations of some heavy metals, particularly cadmium (Cd), mercury (Hg), lead (Pb) and Cu. Furthermore, Pleurotus ostreatus, P. florida, P. sajor-caju, P. eryngii and other white rot fungi have also been found to degrade chlorophenols, lindane, synthetic dyes, PAHs, PCBs (Radha et al., 2005). However, there has not been any investigation on the use of P. ostreatus for mycoremediation of cutting fluid contaminated soil.

The objectives of this study are to assess the influence of P. ostreatus during incubation period on the nutrient content, level of some heavy metal contents, Total Petroleum Hydrocarbon (TPH) loss in the spent and fresh cutting fluids contaminated soil. To also investigate, level of soil fertility using cation exchange analysis after the remediation process.

**MATERIALS AND METHODS**

**Sample location and collection**

**Soil:** The soil used for this experiment was collected from the nursery site of the Department of Botany, University of Ibadan. Top soil was dug at 1 - 10 cm deep using stainless hand trowel and sieved to remove debris.
Fungi: Pure cultures of *Pleurotus ostreatus* were collected from Plant Physiology Laboratory of Department of Botany, University of Ibadan.

Rice Straw: Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (IITA) in Ibadan and air dried in a clear open space for seven days to reduce moisture content. This dehydration process is necessary to prevent decomposition. The dried rice straw had a Golden/Light brown colour. The straw was cut into 0.1 – 3 cm using guillotine.

Wheat Bran: Wheat bran was collected from the feed mill of the popular Bodija Market located in Ibadan.

Cutting Fluid: Fresh and spent cutting fluid were obtained from the Physics Department workshop, University of Ibadan.

Preparation of pure isolates spawn.

The pure spawn was prepared according to the method of Jonathan and Fasidi (2001). The substrate (rice straw) was soaked in water for one hour to moisten the straw and then squeezed using muslin cloth until no water oozed out. Small quantity of wheat bran (additive) was added to the moist straw which was then put into 350 mL sterile bottles, covered with aluminium foil and autoclaved at 151bs pressure and 121 °C temperature for 15min. The bottles were incubated at 28 ± 2 °C for 3 weeks until the substrate was completely ramified to form a spawn.

Experimental set up for culture conditions.

The culture conditions was carried out according to the method of Adenipekun and Fasidi (2005) and modified as follows : Exactly 400 g of soil moistened with 75 % distilled water (w/v) were weighed into 350 mL sterile bottles and then mixed thoroughly with varying concentrations of cutting fluid (0 , 10, 20 and 30 %). A mud balance in Petroleum Engineering Department, University of Ibadan was used to determine the weight percent volume as density (g/ml) of the fluid. A known weight (80 g) of moistened rice straw was laid on the contaminated soil in each bottle separated with a wire gauze and covered with an aluminium foil. Three replicates for each experiment were prepared. The bottles were then autoclaved at 151bs pressure and a temperature of 121 °C for 15 minutes. After cooling, each bottle was inoculated with 10 g of vigorously grown spawn of *Pleurotus ostreatus*. The bottles were incubated at room temperature for two months.

In the first set of control treatment, cutting fluids were not added to the soil but inoculated with fungi while in the second set different levels of oils were added to the soil but no fungal inoculation. At the end of the incubation period, the mycelia ramified substrate was carefully separated.
from the soil layer ensuring that soil particles did not mix with it. All contaminated soils samples were analyzed for physicochemical parameters after drying.

**Physiochemical parameters** Soil pH was measured in soil- water suspension (1: 1) using Jenway model 3510 pH meter ( Bates 1954 ). Organic matter and organic carbon were determined by oxidation with potassium dichromate (K₂Cr₂O₇) and unreacted K₂Cr₂O₇ titrated with standard FeSO₄ (Nelson and Sommers 1982). Total nitrogen was measured by the kjeldahl method, phosphorus was determined colorimetrically by ascorbic acid method and potassium content was measured by flame spectrophotometry (AOAC 2003).

The soil samples were digested with a nitric and mixture of hydrochloric acid (1: 3). Manganese (Mn), Lead (Pb), Nickel (Ni), and Copper (Cu) in the digests were measured by Atomic Absorption Spectrophotometry (AAS). Total Petroleum Hydrocarbon (TPH) was determined by the method of the Inter-governmental Oceanographic Commission (IOC) as described by Onianwa and Essien (1999).

Cation exchangeable capacity (CEC) of soil was calculated from exchangeable cations of K⁺, Na⁺, Ca²⁺, Mg²⁺ and Al³⁺ extracted with 1 M NH₄OAC solution. The K⁺ and Na⁺ concentrations in the extracts were determined by flame spectrophotometry. The Ca²⁺, Mg²⁺ and Al³⁺ concentrations were determined by flame atomic absorption spectrophotometry (Sparks et al., 1996).

**Statistical analysis:** The experiment was carried out in triplicates. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan’s multiple range tests (SPSS 20 version). Differences were considered significant at pd”0.05. Data were also presented with graphical form using mean values with error bars.

**RESULTS**

**Nutrient Contents and pH**
Comparison of nutrient contents of spent cutting fluid with fresh cutting fluid contaminated soil incubated with *Pleurotus ostreatus* for 2 months are presented in Figures 1 to 3. Organic carbon, organic matter contents of spent cutting fluid contaminated soil increased with incubation period while fresh cutting fluid contaminated soil decreased with the incubation period (Figure 1). After 2 months, high organic carbon and organic matter contents were observed at all levels of spent cutting fluid contaminated soil except at 30 % contamination level. Organic carbon and organic matter contents of fresh cutting fluid contaminated soil was higher than that of spent cutting fluid contamination levels and the control treatment level.

Nitrogen content of spent cutting fluid contaminated soil increased with incubation period while that of fresh cutting fluid contaminated soil decreased (Figure 2). Nitrogen content for 30 %
fresh cutting fluid contamination level was higher at 2 months compared with the nitrogen contents for other spent cutting fluid contamination levels even with the control treatment. Also, the potassium content in spent cutting fluid contaminated soil decreased with incubation period while that of fresh cutting fluid contaminated soil increased with the incubation period.

Figure 2 revealed that soil pH decreased after 2 months of incubation for the control treatment and all other levels (10%, 20% and 30%) of soil contamination with both fresh and spent cutting fluid with the incubation period. Phosphorus content generally increased after incubation with the fungus for both fresh and spent contaminated soil (Figure 3).
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Figure 2. pH, Total nitrogen (TN), Potassium (K) contents of spent cutting and fresh cutting fluid contaminated soils incubated with *P. ostreatus* for 2 months.

**Heavy metal contents**

Comparison of heavy metal contents in spent cutting fluid with fresh cutting fluid contaminated soil incubated with *Pleurotus ostreatus* are presented in Figures 4 and 5. Manganese and lead contents decreased with incubation period in spent and fresh cutting fluid contaminated soil samples (Figure 4). It was observed that the levels of Manganese and lead in spent cutting fluid contaminated soil after incubation period were high compared with the corresponding levels in fresh cutting fluid contaminated soil. Also, reduction in nickel and copper contents were observed in spent and fresh cutting fluid contaminated soil samples (Figure 5). Similarly, levels of nickel and copper in spent cutting fluid contaminated soil were higher than the corresponding levels in fresh cutting fluid contaminated soil.

**Total Petroleum hydrocarbons (TPH)**

Total Petroleum Hydrocarbons in spent cutting fluid and fresh cutting fluid contaminated soils are shown in Table 1. TPH contents decreased after the incubation period. TPH loss of 36.8 % was obtained at 10 % contamination level for spent cutting fluid. TPH loss decreased with increased contamination level resulting to...
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Figure 3. Phosphorus (P) contents of Spent and Fresh cutting fluid contaminated soils incubated with *P. ostreatus* for 2 months

TPH loss of 16.2 % at 30 % contamination level (Table 1). TPH loss of 32.1 %, 21.3 % and 15.2 % were obtained at 10 %, 20 % and 30 % of contamination levels for fresh cutting fluid contaminated soil.

Cation exchange capacity (CEC)

The cation exchange capacity for spent and fresh cutting fluid contaminated soil samples incubated with *Pleurotus ostreatus* are shown in Table 2. After two months of incubation, a decrease of 6.51 to 6.20 cmol/kg, 7.43 to 6.99 cmol/kg and 8.96 to 7.08 cmol/kg were recorded for 10 %, 20 % and 30 % respectively for spent cutting fluid contaminated soil when compared with the control (0 %) which showed an increase from 6.21 to 6.98 cmol/kg. No significant difference were observed among the contamination levels (Pd"0.05). Conversely, an increase in CEC of soils contaminated with fresh cutting fluid was observed.

DISCUSSION

The ability of this *P. ostreatus* to grow on cutting fluids contaminated soils at different levels of contamination through their mycelia colonization lends credence to Aust *et al.*
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Figure 4. Manganese (Mn) and Lead (Pb) contents of Spent and Fresh cutting fluids contaminated soil incubated with *P. ostreatus* for 2 months.

(2003) who reported that white rot fungi can withstand toxic levels of most organic pollutants. The increase in nutrient contents incubated with *P. ostreatus* on the spent cutting fluid contaminated soil indicates that mycoremediation has taken place. Adenipekun and Omoruyi (2008) similarly reported high organic carbon and organic matter in bioremediation of cement and battery polluted soil, when compared with control samples. Also, increase in nutrient contents could be attributed to the fact that spent cuttings fluids, would have accumulated more carbon during its use in different metal works due to deposition of CO$_2$ on exposure to atmosphere. There is possibility of the spent cutting fluid losing its potency of inhibiting bacterial growth when in use, which might have made those bacteria perform some degradation aerobically before its use for this studies, thereby, creating a long chain of carbon which was further degraded by the white rot fungus (*P. ostreatus*). This is in accordance to the findings of Rehm and Reiff (1981); Baker and Herson (1990) that aerobic conditions were generally considered necessary for extensive degradation of hydrocarbons in the environment.
However, a reduction in the organic carbon, organic matter in the fresh cutting fluid contaminated soil incubated with *P. ostreatus* also indicates degradation. This is in agreement to the findings of Artuchelvan *et al.* (2003) that decrease in the carbon and nitrogen contents in the soil are due to the degradation process that has been taken place, which further improves soil fertility to the soil thereby altering the C/N ratio of the soil.

A reduction of potassium in spent cutting fluid contaminated soil also shows that degradation has occurred because *P. ostreatus* has accumulated the potassium during the remediation process for synthesis of degradation enzymes and metabolism of the fungus. The reduction in potassium can also be related to a report by Kuforiji (2005) that potassium and magnesium are co-factors in some fungi enzymes system which involved in carbohydrate metabolism and are important in the maintenance of ionic balance.
Table 1: Total petroleum hydrocarbon contents of spent and fresh cutting fluid contaminated soil samples incubated with *Pleurotus ostreatus* for 2 months.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation Period</th>
<th>Spent cutting fluid TPH (mg/kg)</th>
<th>TPH LOSS (%)</th>
<th>Fresh cutting fluid TPH (mg/kg)</th>
<th>TPH LOSS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>0 month</td>
<td>2509&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>10%</td>
<td>0 month</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>1586&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.8</td>
<td>2 months</td>
<td>1952&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>20%</td>
<td>0 month</td>
<td>4096&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>20%</td>
<td>0 month</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>3172&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.6</td>
<td>2 months</td>
<td>3375&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>30%</td>
<td>0 month</td>
<td>5714&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>30%</td>
<td>0 month</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>4790&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2</td>
<td>2 months</td>
<td>5748&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is a mean of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test (p<0.05).

From this study, pH played a major role in the biodegradation process of both spent and fresh cutting fluid contaminated soil samples. The pH reduction indicates that microbial activity took place in the soil after inoculation with *P. ostreatus*. This follows the report by Nwachukwu and Ugoji, (1995); Oboh *et al.* (2006) that microbial degradation of hydrocarbons often leads to production of organic acids coupled with other metabolic products which are factors that account for the reduction in pH levels. Watanabe *et al.* (2012) reported that a pH ranging from 5 to 6 best enhanced the degradation of phenolics and genotoxicity of pharmaceutical effluent by *Pycnoporus sanguineus*. Similar findings by Marandi *et al.* (2010) stated that biosorption of Lead (II) ions in biomass of *Phanerochaete chrysosporium* is at optimum pH of 6. Also, Roushdy (2011) showed that lignin degrading enzyme (LiP) by *Cunninghamella elegans* function best at optimum pH of 6 which enhanced lignin degrading enzyme (LiP).

A reduction in heavy metal contents of the spent and fresh cutting fluid contaminated soils with incubation period indicates that the heavy metals were accumulated by *P. ostreatus*.
Table 2: Cation Exchange Capacity (CEC) (cmol/kg) of spent and fresh cutting fluid contaminated soil samples incubated with *Pleurotus ostreatus* for 2 months.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation period</th>
<th>CEC</th>
<th>Treatment</th>
<th>Incubation period</th>
<th>CEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0%)</td>
<td>0 month</td>
<td>6.21±0.31b</td>
<td>Control (0%)</td>
<td>0 month</td>
<td>6.21±0.31d</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>6.98±1.10b</td>
<td></td>
<td>2 months</td>
<td>6.98±1.10d</td>
</tr>
<tr>
<td>10%</td>
<td>0 month</td>
<td>6.51±0.10b</td>
<td>10%</td>
<td>0 month</td>
<td>5.96±0.06d</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>6.20±0.04b</td>
<td></td>
<td>2 months</td>
<td>6.23±0.06d</td>
</tr>
<tr>
<td>20%</td>
<td>0 month</td>
<td>7.43±0.02b</td>
<td>20%</td>
<td>0 month</td>
<td>6.69±0.26d</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>6.99±0.09b</td>
<td></td>
<td>2 months</td>
<td>8.11±0.36c</td>
</tr>
<tr>
<td>30%</td>
<td>0 month</td>
<td>8.96±0.24b</td>
<td>30%</td>
<td>0 month</td>
<td>8.85±0.12ab</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>7.08±0.28b</td>
<td></td>
<td>2 months</td>
<td>9.51±0.22c</td>
</tr>
</tbody>
</table>

Each value is a mean and standard deviation of three replicates. Values in the same column with different letters as superscripts are significantly different by Duncan multiple range test (*p* < 0.05)

This observation is in agreement to the findings of Gabriel *et al.* (2005) who reported the accumulation of cadmium, lead, aluminium and calcium by wood rot fungi from liquid medium supplemented with appropriate amounts of metal salt. Similarly, Asiriuwa (2013) reported the sclerotia of *Pleurotus tuber-regium* as bioaccumulator of heavy metals from poultry dropping amended soil for which Cu ranging between 10.5 mg/kg and 41.9 mg/kg and cadmium ranging between 3.9 mg/kg and 17.9 mg/kg were best accumulated by the sclerotia. The bioaccumulation order was Cu > Zn > Pb > Cd. Kalac *et al.* (1999); Gadd (2001) also reported the use of fungi in the treatment of effluents containing heavy metal. This was possible due to their ability to accumulate heavy metals from the environment. The ability of Mn to be most
accumulated metal from contaminated soil by *P. ostreatus* could be attributed to its nature of being the most abundant metals in soil (Samuel *et al*., 2010). Similarly, Boamponsem *et al*. (2013) reported that Mn was the second absorbed metal by *P. ostreatus* in metal scrap site. Thus, it is important to note that heavy metals such as Cu, Zn, Fe and Ni accumulated by microbes are micronutrients which are needed for their growth (Bruins *et al*., 2000).

Total Petroleum Hydrocarbon (TPH loss) reduction in spent and fresh cutting fluids contaminated soil is in agreement with the report by Ejoh *et al*. (2012) where the highest total petroleum hydrocarbon (TPH) loss of 36.23 % was recorded at 10 % cutting fluids concentration in unsterilized cutting fluids contaminated soil incubated with *P. tuber-regium* compared to unsterilized cutting fluids contaminated soil which yielded 19.61 % TPH loss. Similarly, the ability of white fungi to degrade petroleum hydrocarbon was reported by Hadibarata and Tachibana (2010) in the use of *Polyporus* sp. S133 to degrade phenanthrene, a polycyclic aromatic hydrocarbon, in a mineral salt broth liquid culture where maximal degradation rate was 92.0 % for 30 days and 44.0 % degradation rate for non-agitated cultures.

The reduction in cation exchange capacity incubated with *P.ostreatus* on the spent cutting fluid contaminated soil was as a result of abundant manganese present in the soil after the remediation of the pollutant coupled with the low pH of the soil, which enables the fungus to perform the mycoremediation process. This was in agreement with the report by Fabio and Reinaldo (2012) that abundance minerals as oxides of metals such as manganese coupled with low pH are the factors responsible for low CEC in soils. Chi and Richard (1999) explained further that CEC of soil is dependent on soil pH value and soil particle size.

An increase in fresh cutting fluids contaminated soil CEC is expected as a result of the degradation of logs positive ions chain in the contaminated soil which would have been broken into smaller elements, suggesting that soil fertility depends on the richness of carbon and nitrogen contents of the soil contaminants and incubation months. This is in agreement with the discoveries of Mandelbaum *et al*. (1993); Alvey and Crowley, (1995); Ojo (2000) on the importance of C and N coupled with activity of a degrading microbial consortium as factors that regulate the rate of mineralization of atrazine contaminated soil.

CONCLUSION.
The experiment carried out reaffirmed the ability of *P. ostreatus* as good mycoremediating agents after inoculation on different levels of spent cutting fluid and fresh cutting fluid on contaminated soil at different incubation periods. This include significant improvement in the nutrient contents of the contaminated soil by increasing the organic matter, organic carbon, nitrogen, phosphorus and potassium as the levels of
the spent cutting fluid and fresh cutting fluid increased. The accumulation of heavy metals such as manganese, copper, lead and nickel showed that *P. ostreatus* is a good bioaccumulator of toxic metals. The change in pH as the incubation period increased also indicated the ability of the fungi to degrade contaminants. The TPH reduction after incubation period revealed the potential of the white rot fungus to mycoremediate organic pollutants. The increased cation exchange capacity revealed high fertility of the soil.

REFERENCES


