Effect of *Pleurotus tuber-regium* Singer and microorganisms on degradation of soil contaminated with spent cutting fluids

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Abstract The ability of *Pleurotus tuber-regium* and indigenous microorganisms isolated from the contaminated soil to remediate spent cutting fluids contaminated soil were investigated for 2months. The ability of these organisms to degrade the total petroleum hydrocarbon present in the cutting fluids, lignin content in the rice straw, their enzyme activity as well as their ability to accumulate heavy metals present in the contaminated soil were monitored. In each of the soil samples (unsterilized cutting fluids contaminated soil (US) and unsterilized cutting fluids contaminated soil inoculated with *P. tuber-regium* (USP)), significant increase (P≤ 0.05) in the nutrient content of the soil was observed; USP having higher nutrient content. The heavy metal contents of the two soil samples decreased showing that bioaccumulation of the heavy metals had occurred. The indigenous microbes alone in US accumulated heavy metals better than the indigenous microbes with *P. tuber-regium* in USP. The highest total petroleum hydrocarbon (TPH) loss (36.23%) was recorded at 10% cutting fluids concentration in USP compared to US which had 19.61%. The unsterilized contaminated soil inoculated with *P. tuber-regium* recorded the highest lignin degradation, also the polyphenol oxidase and peroxidase activity of the organisms in all the samples showed gradual increase. The microorganisms isolated from the unsterilized soil samples were *Bacillus licheniformis, B. cereus, Bacillus sp, Pseudomonas aeruginosa, Pseudomonas sp, Corynebacterium sp, Neisseria sp, Trichoderma harzanium, Aspergillus niger, A. flavus, A. glaucus, Mucor sp* and *Rhizopus sp.*

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1. Introduction

Cutting fluids are special blend of chemical additives, lubricants and water formulated to meet the performance demand of the metalworking industry (Mackerer *et al*., 1995). They are made up of hydrocarbons, fatty acids, emulsifiers, organo-corrosion inhibitors, amines and glycols but they may also contain organo-boron complexes, copolymer, esters, alcohol, halogenated hydrocarbon, organophosphate and phosphate (Gannon *et al*., 1981). There are four types of cutting fluids; straight oil, synthetic fluids, soluble oil and semi-synthetic fluids (Aronson, 1994; Bienkowski, 1993). Spent soluble oil was used as the contaminated in the present study because of its composition (petroleum oil, chemical additives and emulsifiers) and heavy metal constituents all of which are hazardous to living organisms in the environment.

Bioremediation is a means of cleaning up contaminated environments by exploiting the diverse metabolic activities of microorganisms to convert the contaminants to harmless products by mineralization, generating carbon IV oxide and water or by conversion into microbial biomass (Mentzer and Ebere, 1996). Biological agents such as bacteria, fungi, algae and other green plants have been reported to be involved in bioremediation process and this they do by producing enzymes, biosurfactants, biopolymers and other fermentative products.

White rot fungi have been known to degrade lignin and other xenobiotic pollutants (Crawford, 1981). *Pleurotus tuber-regium* which is an edible white rot fungus have been reported to be involved in the remediation of hydrocarbon pollutants and bioaccumulation of heavy metals. Adenipekun *et al*., 2011 investigated the bioaccumulation of heavy metals and nutrient content supplementation by two white rot fungi *P. ostreatus* and *P. pulmonarius* in crude oil polluted soils after 1 and 2months of incubation. They reaffirm that these two white rot fungi are good bioremediating and bioaccumulating agent for any hydrocarbon polluted soil.

Microorganisms such as *Pseudomonas sp, Bacillus sp, Corynebacterium sp, Trichoderma sp Rhizopus sp, Mucor sp* and *Aspergillus sp* have also been exploited in the remediation of organo-pollutants (Obire *et al.,* 2008).The focus of this work was to investigate the ability of *P. tuber-regium* and the microbes present in the unsterilized soil sample to remediate the cutting fluids polluted soil.

2. Materials and Methods

Collection of samples

Cutting fluids was obtained from the Workshop of the Department of Physics, University of Ibadan. The soil was collected from the Nursery site of the Department of Botany, University of Ibadan, sieved with a 2mm mesh, then dispensed into sterile bottles and taken to the laboratory. Freshly harvested rice straw (fungus substrate) was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, and sun dried for seven days to reduce its moisture content. After drying, the straw was cut into smaller sizes (0.1 – 2cm), using a guillotine. The wheat bran which served as an additive was obtained from Bodija market, Ibadan. Pure culture of *Pleurotus tuber-regium* was collected from the Plant Physiology Laboratory of the Department of Botany, University of Ibadan. The microbial species (bacteria and fungi) were isolated from the contaminated soil.

Spawn preparation

This was done using the method of Jonathan and Fasidi (2001).

Experimental Setup

The method of Adenipekun and Fasidi (2005) was used. Soil sample (200g) was weighed into sterile bottles and this was thoroughly mixed with varying concentrations of cutting fluids (5, 10, 20 and 30%). Moist clean rice straw (40g w/v) was laid on the contaminated soil in each bottle, separated by wire gauze and covered with aluminum foil. Thereafter, each bottle was inoculated with 10g of vigorously growing spawn of *P. tuber-regium*. All the bottles were incubated at room temperature for 2 months at 28 $\pm$ 2^0 C in an incubator. Each treatment was in three replicates. In the control, cutting fluids was not added and another set which *P. tuber-regium* was not introduced. 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 the substrate. The soil samples were analyzed for physico-chemical parameters after drying.

Isolation and Characterization of microorganisms

After incubation, the bacterial species in the cutting fluids contaminated soil samples were isolated by pour plate technique using 0.1 ml aliquots of appropriate dilution into nutrient agar plates. Individual cultures were identified by morphological and biochemical techniques using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

The fungi species indigenous to the cutting fluids contaminated soil were isolated by plating out the diluents on Potato Dextrose Agar (PDA) into which streptomycin (50 mg/ml) had been added to suppress bacterial growth. Fungal isolates were identified using morphological appearance/microscopic observation and comparing with pictures documented in the compendium of soil fungi (Domsch *et al*., 1980).

The bacteria and fungi cultures obtained were screened for their ability to utilize cutting fluids as carbon source for growth.

Determination of soil pH

The pH was determined by the method of Bates (1954).

Nutrient content analysis

The organic matter, organic carbon, percentage nitrogen, phosphorus, potassium and calcium contents were determined using the method described by the Association of Official Analytical Chemists (A.O.A.C., 2003).

Determination of lignin content of rice straw

The method of Southgate (1967) and Van Soest and Wine (1968) were used in the determination of the lignin content of the rice straw.

Determination of Total Petroleum Hydrocarbon (TPH)

This was done according to the modified method of Osuji and Nwoye (2007).

Determination of heavy metals in soil

The heavy metal content of the soil was determined by the method of Crosby, 1977.

Determination of enzyme activity

This was done using the method of Maxwell and Bateman (1967).

Statistical analysis

A randomized factorial experiment showing unsterilized cutting fluids polluted soil (US) and unsterilized cutting fluids polluted soil inoculated with *P. tuber-regium* (USP) incubation periods were 0 and 2months was done. An ANOVA table was prepared for the data obtained followed by the Duncan's multiple range test for the separation of means.

3. Results

The microorganisms isolated from the unsterilized soil samples were *Bacillus licheniformis, B. cereus, Bacillus sp, Pseudomonas aeruginosa, Pseudomonas sp, Corynebacterium sp, Neisseria sp, Trichoderma harzanium, Aspergillus niger, A. flavus, A. glaucus, Mucor sp* and *Rhizopus sp*. The isolates were also found to grow on 5 % and10 % cutting fluids as carbon source.

Table 1 shows the percentage TPH loss in US and USP after the remediation process for 2months. The unsterilized soil inoculated with *P. tuber-regium* (USP) showed the highest % TPH loss (21.37%) at 5% cutting fluids contamination, 36.23% at 10% cutting fluids contamination. Also at 20% cutting fluids contamination, USP recorded highest % TPH loss of 29.53% except at 30% contamination where the TPH loss was low and

this may be due to exhaustion of the TPH in the soil, indicating that the indigenous microbes and the mushroom were more efficient in degrading the cutting fluids. In US where the indigenous microbes alone were left to degrade the cutting fluids, the % TPH loss were 19.77% at 5% contamination, 19.61% at 10% contamination, 12.95% at 20% contamination and 17.55% at 30% cutting fluids contamination.

The heavy metal content of the unsterilized soil (US) and unsterilized soil inoculated with *P. tuber-regium* (USP) for 2months is shown in Table 2. Increase in the heavy metal contents for both soil samples were observed except for nickel which increased at 0% and 5% cutting fluids contamination but decreased from 0.20- 0.14mg/kg for US and to 0.04mg/kg in USP at 10% cutting fluids contamination. The Ni content also decreased at 20% and 30% cutting fluids contamination, indicating that Ni was better accumulated by the organisms than all other heavy metals.

Table 3 shows the nutrient contents of the different soil samples incubated for 2months. Unsterilized soil inoculated with the mushroom performed better than US in improving the nutrient content of the soil. The organic carbon and organic matter in the unsterilized soil incubated with *P. tuber-regium* (USP) increased from 6.17% at 0% to 18.63 at 10%, later to 28.37% at 30% cutting fluids contamination and 10.63% at 0% to 32.13% at 10% and later to 48.73% at 30% cutting fluids contamination respectively.

The N, P, Ca and K also increased but the increase was gradual, from 0.60% at 0% to 1.77% at 10% cutting fluids contamination and later to 2.25% at 30% cutting fluids concentration, from 5.88mg/kg at 0% cutting fluids concentration to 14.55mg/kg at 10% cutting fluids concentration and later to 20.80mg/kg at 30 % cutting fluids contamination, from 0.497cmool/kg at 0% cutting fluids concentration to 1.180cmol/kg at 10% cutting fluids concentration and later to 2.20cmol/kg at 30% cutting fluids concentration and from 0.640cmol/kg at 0% cutting fluids concentration to 1.59cmol/kg at 10% cutting fluids concentration and later to 1.97cmol/kg at 30% cutting fluids contamination respectively. A similar trend was observed in US.

The pH of the different samples reduced compared to the control at all levels of spent cutting fluids contamination, with the pH value ranging from 5.25-6.567.

Fig 1 shows the loss of lignin content in the rice straw present in US and USP incubated for 2months. The lignin content reduced from 34.50-18.53% at 0% contamination, from 34.40-12.03% at 5% contamination, from 32.53-8.39% at 10% contamination, from 32.90-6.10% at 20% contamination and from 32.67-4.70% at 30% cutting fluids contamination. A similar trend was observed in US.

Fig 2 shows the polyphenol oxidase activity of the organisms present in the different soil samples. The indigenous microbes and the mushroom in USP had higher polyphenol oxidase activity than the microbes alone in US. As the concentration of cutting fluids increased, the enzyme activity also increased indicating that the organisms were able to utilize the cutting fluids and the rice straw as nutrient, the activity of the enzyme increased from 0.25unit/ml at 0% contamination to 0.29unit/ml at 5%, 0.40unit/ml at 10%, 0.51unit/ml at 20% and finally to 0.59unit /ml at 30% cutting fluids contamination.

The peroxidase activity of the different soil samples incubated for 2months is shown in Fig 3, the highest peroxidase activity was also recorded in USP from 0.25unit/ml at 0% cutting fluids contamination to 0.27unit/ml at 10% and then to 0.65unit/ml at 30% cutting fluids contamination. It is worthy to note the peroxidase activity of these organisms is higher than the polyphenol oxidase activity.

Table 1. Total Petroleum Hydrocarbon contents in unsterilized soil spent with unsterilized cutting fluids incubated for 2 months with and without *P. tuber-regium*

Treatment (Conc. of CF)	0 month	US	TPH (% loss)	USP	TPH (% loss)
5%	8.75±0.006^{d}	7.02±0.012^{d}	19.77	6.88±0.034^{d}	21.37
10%	11.37±0.006^{c}	9.14±0.009^{c}	19.61	7.25±0.040^{c}	36.23
20%	13.75±0.006^{b}	11.97±0.006^{b}	12.95	9.69±0.010^{b}	29.53
30%	15.61±0.006^{a}	12.87±0.006^{a}	17.55	14.81±0.010^{a}	5.12

Each value is a mean of three replicates. Values in the same column followed by different letters are significantly different according to Duncan multiple range test at (P≤ 0.05).

Key: US- Unsterilized soil contaminated with spent cutting fluids

USP- Unsterilized spent cutting fluids contaminated soil inoculated with *P. tuber-regium*

Table 2. Heavy metal content (mg/kg) of soil contaminated using unsterilized cutting fluids incubated for two months with and without *P. tuber- regium*

Treatment (Conc. of cutting fluids)	Months	Mn	Cu	Pb	Zn	Fe	Ni
0%	Control	0.27±0.009^{c}	0.37±0.009^{b}	0.23±0.024^{b}	0.16±0.003^{c}	0.62±0.006^{b}	0.10±0.003^{c}
	US	0.35±0.006^{b}	0.38±0.009^{b}	0.23±0.010^{b}	0.22±0.006^{b}	0.58±0.009^{c}	0.16±0.000^{b}
	USP	0.40±0.006^{a}	0.45±0.006^{a}	0.33±0.006^{a}	0.68±0.006^{c}	0.67±0.009^{a}	0.18±0.000^{a}
5%	Control	0.32±0.006^{c}	0.44±0.006^{c}	0.36±0.006^{c}	0.31±0.006^{c}	0.67±0.009^{c}	0.15±0.007^{b}
	US	0.52±0.009^{b}	0.67±0.006^{b}	0.44±0.009^{b}	0.51±0.006^{b}	0.91±0.009^{b}	0.17±0.006^{b}
	USP	0.62±0.006^{a}	0.76±0.009^{a}	0.63±0.007^{a}	0.60±0.006^{a}	0.95±0.006^{a}	0.29±0.007^{a}
10%	Control	0.50±0.006^{c}	0.67±0.006^{c}	0.47±0.021^{c}	0.37±0.006^{d}	0.80±0.006^{c}	0.20±0.010^{a}
	US	0.74±0.006^{b}	0.76±0.006^{b}	0.60±0.014^{b}	0.69±0.006^{b}	1.86±0.009^{b}	0.14±0.000^{b}
	USP	0.86±0.006^{a}	0.82±0.009^{a}	0.67±0.003^{a}	0.73±0.006^{a}	1.94±0.009^{a}	0.04±0.000^{c}
20%	Control	0.65±0.006^{c}	0.70±0.006^{c}	0.59±0.012^{b}	0.55±0.009^{c}	0.93±0.006^{c}	0.34±0.009^{a}
	US	1.57±0.006^{b}	1.21±0.006^{b}	0.74±0.003^{a}	0.81±0.009^{b}	1.93±0.006^{b}	0.12±0.006^{c}
	USP	1.68±0.006^{a}	1.45±0.009^{a}	0.72±0.020^{a}	0.85±0.006^{a}	2.15±0.006^{a}	0.10±0.000^{d}
30%	Control	0.81±0.006^{c}	0.77±0.006^{c}	0.94±0.009^{a}	0.64±0.006^{c}	1.15±0.006^{c}	0.42±0.006^{a}
	US	1.95±0.006^{b}	1.77±0.009^{b}	0.86±0.009^{b}	1.95±0.006^{b}	1.98±0.009^{b}	0.10±0.000^{c}
	USP	2.51±0.006^{a}	1.81±0.006^{d}	0.88±0.035^{b}	2.17±0.009^{a}	2.24±0.009^{a}	0.080±0.000^{d}

Each value is a mean of three replicates. Values in the same column followed by different letters are significantly different according to Duncan multiple range test at (P≤0.05).

Key: US- Unsterilized soil contaminated with cutting fluids

USP- Unsterilized cutting fluids contaminated inoculated with *P. tuber-regium*

Table 3. Nutrient content of soil contaminated with spent cutting fluids incubated for 2 months with and without *P. tuber-regium*

Treatment (Conc. of cutting fluids)	Types of sample	OC (%)	OM (%)	N (%)	P (mg/kg)	Ca (cmol/kg)	K (cmol/kg)	pH
0%	Control	8.333^{a}	14.37^{a}	0.833^{a}	5.980^{b}	0.430^{b}	0.490^{c}	6.367^{a}
	US	6.850^{b}	11.81^{b}	0.660^{c}	6.767^{a}	0.403^{b}	0.547^{b}	5.427^{c}
	USP	6.167^{c}	10.63^{c}	0.603^{d}	5.880^{b}	0.497^{a}	0.640^{a}	6.000^{b}
5%	Control	9.730^{d}	16.78^{d}	0.683^{b}	6.537^{d}	0.580^{d}	0.573^{d}	6.353^{a}
	US	12.38^{b}	21.35^{b}	1.397^{a}	10.55^{b}	0.753^{b}	0.870^{b}	5.267^{b}
	USP	14.40^{a}	24.94^{a}	1.403^{a}	11.93^{a}	0.853^{a}	0.967^{a}	6.133^{a}
10%	Control	11.44^{d}	19.72^{d}	1.337^{b}	8.683^{d}	0.723^{d}	0.850^{d}	6.567^{a}
	US	16.63^{b}	28.67^{b}	1.693^{a}	12.92^{b}	1.070^{b}	1.404^{b}	5.250^{d}
	USP	18.63^{a}	32.13^{a}	1.773^{a}	14.55^{a}	1.180^{a}	1.597^{a}	6.167^{b}
20%	Control	13.88^{d}	23.83^{d}	1.330^{c}	9.980^{d}	0.843^{a}	0.863^{c}	6.267^{a}
	US	19.87^{b}	34.25^{b}	2.047^{a}	15.13^{b}	1.677^{a}	1.647^{ab}	6.023^{a}
	USP	21.77^{a}	37.52^{a}	2.143^{a}	17.90^{a}	1.860^{a}	1.717^{a}	6.133^{a}
30%	Control	15.79^{d}	27.22^{c}	1.473^{c}	12.84^{d}	0.907^{c}	0.947^{c}	6.367^{a}
	US	26.10^{b}	48.33^{a}	2.200^{b}	18.27^{b}	1.760^{b}	2.017^{a}	6.003^{c}
	USP	28.37^{a}	48.73^{a}	2.250^{a}	20.80^{a}	2.203^{a}	1.970^{a}	6.300^{b}

Each value is a mean of three replicates. Values in the same column followed by different letters are significantly different according to Duncan multiple range test at (P≤ 0.05)

Key: US- Unsterilized soil contaminated with cutting fluids

USP- Unsterilized cutting fluids contaminated inoculated with *P. tuber-regium*

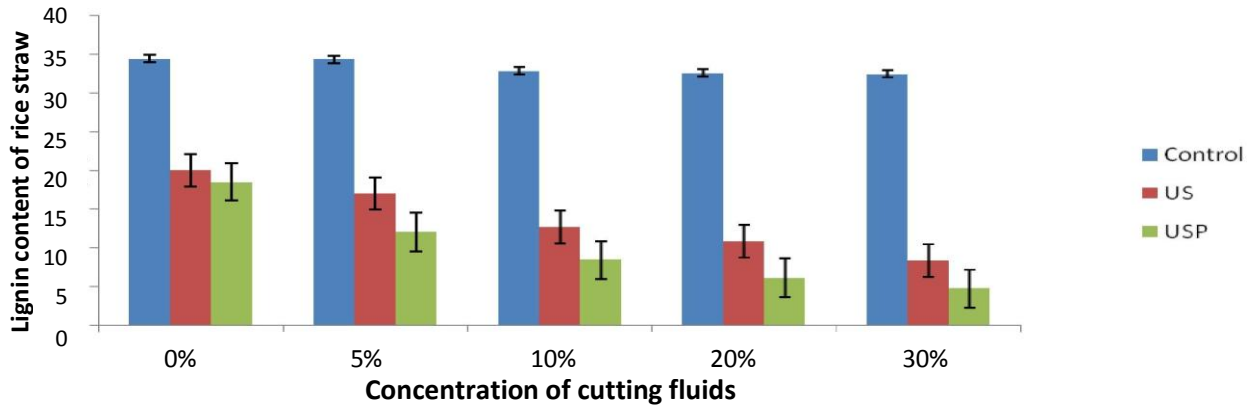

Fig. 1 Reduction in lignin content of rice straw in unsterilized spent cutting fluids contaminated soil incubated with and without *P.tuber-regium* for 2 months
Key: US- Unsterilized soil contaminated with cutting fluids
USP- Unsterilized cutting fluids contaminated inoculated with *P. tuber-regium*

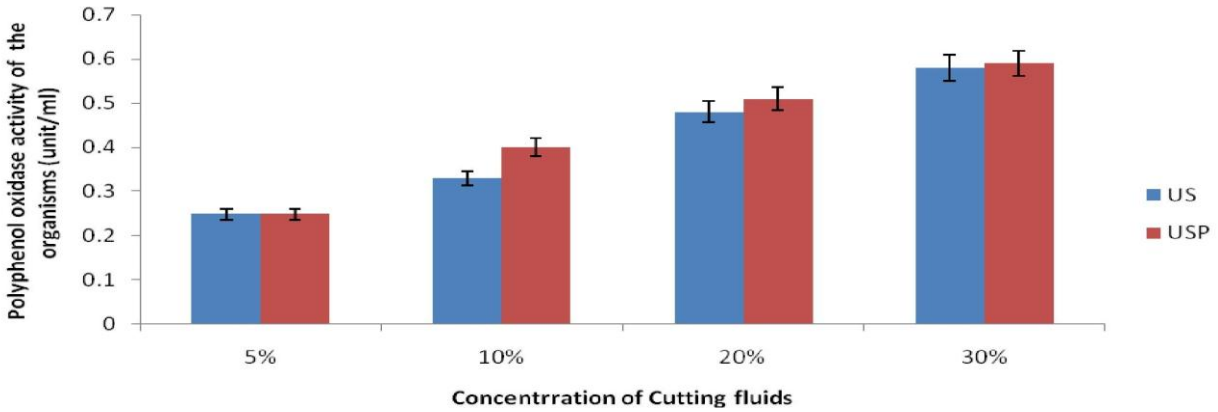

Fig. 2. Polyphenol oxidase activity in unsterilized spent cutting fluids contaminated soil incubated with and without *P. tuber-regium* for 2 months
Key: US- Unsterilized soil contaminated with cutting fluids
USP- Unsterilized cutting fluids contaminated inoculated with *P. tuber-regium*

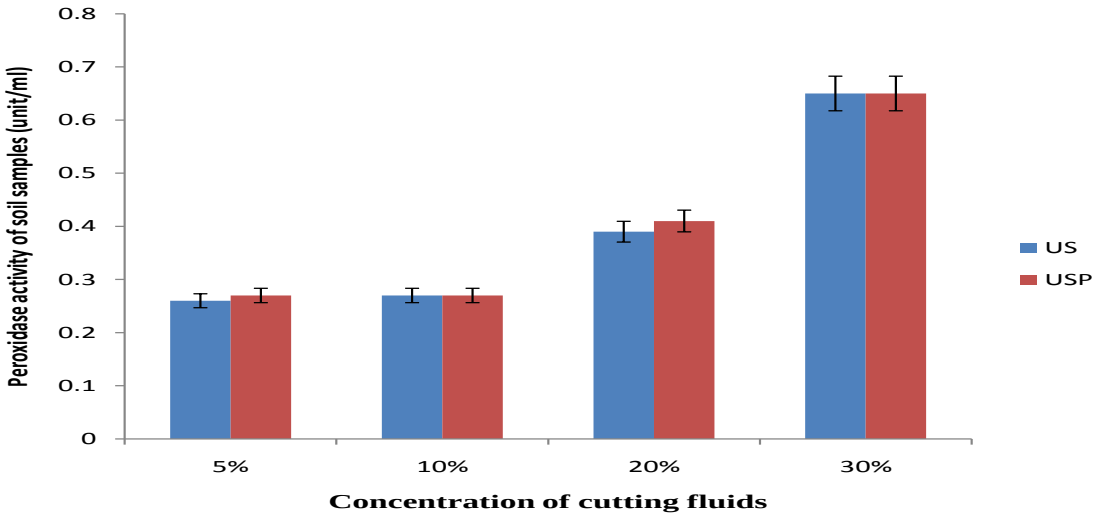

Fig. 3. Peroxidase activity in unsterilized cutting fluids contaminated soil incubated for 2months with and without *P. tuber-regium*
Key: US- Unsterilized soil contaminated with cutting fluids
USP- Unsterilized cutting fluids contaminated inoculated with *P. tuber-regium*

4. Discussion

At all levels of cutting fluids concentration there were significant improvement in the organic carbon, organic matter, phosphorus, potassium, calcium and nitrogen of the different soil samples after 2months of incubation. As observed, the indigenous microbes and the mushroom in USP improved the nutrient content of the soil better than the indigenous microbes alone in US. There was increase in the nutrient contents of the soil with increasing concentrations of the cutting fluids. This is similar to the findings of Adenipekun and Lawal (2011), where increase in nutrient content of palm kernel sludge contaminated soil incubated with *Pleurotus pulmonarius* for 2months was observed to increase with incubation period.

The reduction in the pH of all the soil samples with increase in incubation period conforms with the findings of Adenipekun and Fasidi (2005) that the pH of the soil reduced from 6.90 to 6.62 and eventually to 6.25 after 3 and 6 months respectively. The pH range of the different soil samples (US and USP) incubated for 2 months is 5.25-6.30.

The heavy metal contents of the soil samples reduced as incubation period increased, indicating that the heavy metals were accumulated unto the straw/ fungal/bacterial biomass. There was increase in the heavy metal content of the soil as the concentration of cutting fluids increased showing that the contaminant was rich in heavy metals. In comparing the different soil samples, the indigenous microbes in US showed better ability in accumulating heavy metals. This observation is similar to the findings of Gabriel *et al*. (2005) who reported the accumulation of cadmium, lead, aluminum and calcium by wood-rotting fungi from liquid medium supplemented with appropriate amounts of metal salt. Gadd (2001) and Kalac *et al*. (1999) also reported fungi in the treatment of effluents containing heavy metals due to their ability to accumulate heavy metals from the environment. Pan-Hou and Imura (1982) observed the use of *Pseudomonas aeruginosa* and *Bacillus licheniformis* in the uptake and conversion of metals into less toxic and volatile forms. Chua *et al*. (2000) also stated the involvement of microorganisms in the removal of heavy metals from the environment. It is important to note that some of these heavy metals accumulated by these microbes are micronutrients which are needed for their growth e.g. Cu, Zn, Fe, Ni etc (Bruins *et al*. 2000).

The present study showed a decrease in the TPH content of the soil as incubation period increased indicating that the organisms were able to degrade the TPH in the cutting fluids, but TPH of the soil increased as the concentrations of cutting fluids increased indicating that the contaminant had hydrocarbon content. For the different soil samples, the indigenous microbes and the mushroom in USP showed better degradative ability having the highest TPH loss of 36.23% at 10% cutting fluids concentration.

Rosado and Pitchel (2004) also reported that oil was removed in sunflower/mustard treatment after 150days. In the opinion of Aust *et al*. (2003) white rot fungi have the ability to tolerate toxic levels of most organopollutants. Stamets (1999) observed the use of *Pleurotus sp* in reduction of more than 95% PAH to non-toxic components in the soil. The white rot fungus *Pleurotus ostreatus* was able to remove 86% of the total of 16 PAHs present in creosote contaminated soil (Eggen, 1999).

The lignin content of the rice straw was observed to be reducing with increase in incubation period indicating that some of the indigenous microbes and the mushroom were able to produce lignolytic enzymes to degrade the lignin. This is similar to the findings of Pointing *et al*. (1998) who reported that the ability of fungi to degrade lignocellulose is attributed to their possession of extracellular enzymes mainly lignin peroxidase, manganese peroxidase and laccase.

The polyphenol oxidase and peroxidase activity of the organisms present in all the samples were observed to be increasing with increase in incubation period indicating that the organisms were producing more enzymes in order to degrade the rice straw as well as the cutting fluids whose concentration was increasing. Increased enzyme activity was observed in the present study and this could be as a result of the high percentage of organic matter in the soil. In both soil samples, the peroxidase activity was higher than the polyphenol oxidase activity.

The microbes isolated from the unsterilized samples appeared to have utilized the cutting fluids for their growth and so they were responsible for the degradation of the cutting fluids. This in line with the report of several authors on the use of these isolates for the remediation of oil polluted soils; *Pseudomonas sp* by Leung *et al*. (1997), *Bacillus sp* (Okparanma *et al*. 2009), *Aspergillus sp, Mucor sp, Trichoderma sp* (Obire *et al*., 2008) and *Rhizopus sp*. Oudot *et al*. (1993) also reported some of these fungi (*A. niger, A. flavus, Trichoderma sp, Mucor sp* and *Rhizopus sp*) as hydrocarbon degraders. The abilities of bacterial species; *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Citrobacter* and fungal species which include; *Aspergillus* sp., *Penicillum, Rhizopus* and *Rhodotorula* species to grow on crude petroleum as the sole carbon and energy source when

screened for hydrocarbon utilisation was reported by Oboh *et al*., 2006. Nkwelang *et al*. (2008) reported that the major genera of bacteria active in polluted soils were *Pseudomonas*, *Bacillus*, *Serratia* and *Acinetobacter*, while fungal genera were *Aspergillus*, *Penicillum* and *Mucor*. Santos *et al*. (2008) reported the ability of *Aspergillus* sp. to biodegrade gasoline.

Conclusion

This study established the fact that *P. tuber-regium* and the microorganisms in the contaminated soils had the potential of degrading the lignin content of the rice straw by the production of lignolytic enzymes, degrading TPH in the contaminant, improving the nutrient content of the soil and to accumulate heavy metals present. The indigenous microbes and the mushroom in USP seemed to have been more effective and produced better results than the indigenous microbes alone in US except for heavy metal accumulation where the indigenous microbes in US accumulated them better.

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