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Full Length Research paper

Effect of crude oil contamination on the yield and chemical composition of *Pleurotus tuber-regium* (Fr.) Singer

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The effect of crude oil contamination which is a perennial problem in the Niger Delta of Nigeria on the yield and nutrient status of *Pleurotus tuber-regium* was investigated. In this study, after cultivating *P. tuber-regium* in crude oil contaminated soils to which sawdust, shredded banana leaf blades, NPK fertilizer and poultry litter were added, the yield and nutrient or chemical composition were determined. Crude oil contamination caused significant increase in the size and yield of the mushroom by increasing the pileus and stipe size and also fresh and dry weights showing a fertilizer effect. The addition of sawdust and poultry litter enhanced the fertilizer effect by further significant increases in size and yields of treatments that had them. The addition of NPK and shredded banana leaf blades to crude oil contaminated soils did not enhance the fertilizer effect of crude oil as there was significant decrease in the size and yield of *P. tuber-regium* in treatments that had them. The nutrient chemical composition of the mushroom was also affected by the presence of the crude oil and supplementation with the various materials. There was a reduction in the moisture and carbohydrate content caused by the addition of poultry litter and sawdust to the contaminated soils and an increase in the ash, fat, protein and fibre content of the mushroom. On the other hand, the addition of NPK and shredded banana leaf blades caused a reduction in the moisture, protein and carbohydrate content and an increase in the fat, moisture, ash and fibre content. The performance of the fungus in crude oil contaminated substrates can be optimized by the addition of sawdust and poultry litter but not shredded banana leaf blades and NPK. This is evident from the fact that sawdust and poultry litter enhanced growth while shredded banana leaf blades reduced growth in crude oil contaminated soil. The improvement in nutrient status of the mushroom indicates that the fertilizer effect of crude oil also affects the general well being of the fungus.

Key words: Crude oil contamination, *Pleurotus tuber-regium*, fertilizer effect, nutrient status.

INTRODUCTION

Crude oil contamination of soils is a perennial problem in the Niger Delta, Nigeria. There have been several researches on the biological approach to the clean ups of the spills that have become a regular occurrence. White rot fungi have been used experimentally for the decontamination of petroleum hydrocarbon contaminated soils. Several fungi like *Pleurotus ostreatus*, *Lentinus subnudus* and *Pleurotus tuber-regium* have been grown in crude oil contaminated soils (Isikhuemhen et al., 2003; Adenipekun and Fasidi, 2005; Ogbo and Okhuoya, 2008). Ogbo and Okhuoya (2008) showed that *P. tuber-regium* was able to decontaminate crude oil contaminated soils reducing the various petroleum hydrocarbons in crude oil to varying degrees. The contaminants instead of reducing the growth of the fungus improved their growth. The ability of white rot fungi to degrade petroleum hydrocarbons can be due to the fact that these fungi are

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uniquely equipped as soil remediation agents (Reddy, 1995). White rot are filamentous organisms and as such have the propensity to extend through soil in search of new substrates to exploit and thus colonize larger surface area than bacteria. Furthermore, with the aid of their highly oxidative lignin degrading systems, they are able to oxidize extremely hydrophobic substrates (Kirk and Farrel, 1987).

The only drawback here is that these environmental pollutants or their degradation products may inhibit their lignin degrading system (Fenn and Kirk, 1979; Bumpus and Tatarko, 1994). Petroleum hydrocarbons have been shown to improve the growth of white rot fungi in contaminated soils by increasing their size and yield indicating that the contaminants have a fertilizer effect (Isikhuemhen et al., 2003; Stamets, 2005). The growth of white rot fungi in soil can also be enhanced by the addition of sawdust or other cellulosic wastes to soil matrix resulting in better soil augmentation (Schmidt et al., 2008). The addition of fertilizers is justified from the fact that it has been reported that they are able to improve the biodegradation process (Braddock et al., 1997). The test fungus *P. tuber-regium* is a sclerotium producing one found growing abundantly in the wild, in Nigeria (Isikhuemhen et al., 1999). The success of white rot fungi in crude oil contaminated sites is well documented but the effect on the chemical composition of this hitherto edible mushroom with reference to its nutrient status is not discussed. This investigation therefore aims at finding out what effect the pollutant has on the growth parameters of the mushroom and chemical nutrient status. The effect soil augmentation has on the growth of the fungus in crude oil contaminated soils is also investigated with reference to the nutrients too.

MATERIALS AND METHODS

P. tuber-regium sclerotia and spawn 'mushroom seeds' were inoculated into crude oil contaminated soils. The sclerotia used were bought from a local market in Benin, Nigeria and the spawn was gotten from fruit bodies after sclerotial induction. The methods used for the preparation of spawn are adopted from Ogbo and Okhuoya (2008). The crude oil contaminated soils used for this study were collected from a spill site at Uvwiamughe near Ughelli, Delta State, Nigeria, the same site used in Ogbo and Okhuoya (2008). Top soils (0 - 10 cm) collected from the spill site were supplemented with sawdust, shredded banana leaf blades, poultry litter and NPK fertilizer.

Particle size analysis and substrate composition are as described also in Ogbo and Okhuoya (2008). The various soil amendments or augmentations which made up the substrate combinations were as follow: Control (no crude oil contamination); contaminated soil only: sawdust + contaminated soil; poultry litter + contaminated soil; sawdust + poultry litter + contaminated soil; banana leaf blades + contaminated soil; sawdust + NPK + contaminated soil; banana leaf blades + NPK + contaminated soil; NPK + contaminated soil.

The weight of soil used for each treatment was 1700 g + 300 g of each cellulosic waste for example sawdust or banana leaf blades. In the case of treatments without cellulosic wastes, 1700 g of contaminated soil was added to 300 g of uncontaminated soils and then 40 g of either poultry litter or NPK was added.

Nutrient analysis

The fruit bodies of *P. tuber-regium* were harvested after seven days' growth and dried in an oven at 60°C to constant weight ground in a blender and kept under refrigeration at 4°C. The analysis was carried out in triplicate and consisted of moisture, total fat, total carbohydrates, ash, total protein and crude fibre. The analysis for moisture, total fat, total carbohydrates and ash were determined using AOAC (1985) methods. Moisture of harvested mushroom fruit bodies was determined by drying in a moisture determination apparatus (Preciss, HA60) at 110°C until circulation was complete. Ash was gotten from incinerated residue at 550°C after 3 h and the fat was gravimetrically obtain after Soxhlet extraction with petroleum ether. The total protein was determined from the total nitrogen content using the correction factor 4.38 as stated in Breene (1990).

The experiment had six replicates with nine treatments (substrate combinations) and two variables which were the types of inocula used that is sclerotium and spawn. Standard error values were calculated as well as one way ANOVA with Duncan's multiple tests, used to identify where there were significant differences between variables and the treatments. The SPSS 13.00 for windows was used for this statistical analysis.

RESULTS AND DISCUSSION

Crude oil contamination had a fertilizer effect on the mushroom *P. tuber-regium* as there was significant increase in the size of pileus and stipe and yield shown by increased fresh and dry weights in the presence of the contaminant. The fertilizer effect of petroleum hydrocarbons on white rot fungi had been previously recorded by Stamets (2005) for *P. ostreatus*, with diesel fuel contamination which significantly increased the size and yield of the mushroom. The addition of sawdust and poultry litter enhanced the fertilizer effect by causing further increase in size and yield of the mushroom (Tables 1 and 2). The biggest mushrooms were harvested from substrates with sawdust and poultry litter as indicated by the sizes of the pilei and stipes (Tables 1 and 2). The addition of shredded banana leaf blades and NPK on the other hand, reduced the fertilizer effect of the crude oil resulting in smaller fruit bodies and also no fructification. The adverse effect of NPK fertilizer on the growth of *P. tuber-regium* had been reported previously by Isikhuemhen et al. (1999). The yield followed the same trend as there was higher yield in crude oil contaminated soils/substrates than the control soils that is soils without crude oil contamination (Tables 1 and 2). The highest yields were recorded in crude oil contaminated substrates that had poultry litter and sawdust. The least yields and no fructification were recorded in substrates that had shredded banana leaf blades and NPK (Tables 1 and 2). The yield of *P. tuber-regium* in this study therefore varied with the substrate combination used. This agrees with the work of Liu et al. (2005) who worked on *P. ostreatus* using different substrate combinations. They

Table 1. Effect of crude oil contamination on size and yield of Pleurotus tuber-regium using spawn inoculum.

Substrate composition	Pileus diameter	Stipe height	Stipe girth	Fresh weight	Dry weight
Control	6.53 ± 0.46^{a}	7.68 ± 0.27^{a}	3.18 ± 0.08^{a}	117.84 ± 6.80^{a}	22.03 ± 0.64^{a}
Contaminated soil only	8.44 ± 0.46^{a}	9.74 ± 0.85^{a}	4.09 ± 0.26^{a}	127± 4.27^{a}	23.19 ± 0.46^{a}
Sawdust + contaminated soil	13.85 ± 0.94^{c}	10.24 ± 1.11^{a}	5.16 ± 0.23^{b}	241.84 ± 15.98^{c}	48.51± 4.99^{b}
Poultry litter + contaminated soil	15.70 ± 0.99^{c}	11.30 ± 1.04^{b}	4.92 ± 0.39^{b}	279.84 ± 18.36^{c}	50.39 ± 5.41^{c}
Sawdust + poultry litter + contaminated soil	11.81 ± 0.82^{b}	10.14 ± 0.83^{a}	3.76 ± 0.23^{a}	112.75 ± 15.27^{a}	29.71 ± 3.60^{a}
Banana leaf blades + contaminated soil	2.56 ± 1.23^{d}	1.98 ± 0.94^{c}	1.07 ± 0.51^{c}	71.90 ± 0.00^{e}	6.38 ± 0.00^{d}
Sawdust + NPK + contaminated soil	0.72 ± 0.49^{d}	1.39 ± 0.87^{c}	0.67 ± 0.42^{c}	32.52 ± 0.00^{e}	1.24 ± 0.00^{d}
Banana leaf blades + NPK + contaminated soil	0.78 ± 0.50^{d}	1.37 ± 0.87^{c}	0.64 ± 0.41^{c}	30.25 ± 0.00^{e}	2.84 ± 0.00
NPK + contaminated soil	-	-		-	-

Numbers [a] (means ± standard error of differences) within each column followed by same letters are not significantly different at P ≤ 0.05 (n = 6) Duncan's multiple range test.

Table 2. Effect of crude oil contamination on size and yield of Pleurotus tuber-regium using sclerotium inoculum.

Substrate composition	Pileus diameter	Stipe height	Stipe girth	Fresh weight	Dry weight
Control	7.21 ± 0.45^{a}	8.92 ± 0.43^{a}	3.00 ± 0.06^{a}	158.61 ± 10.73^{b}	29.86 ± 1.32^{a}
Contaminated soil only	10.99 ± 0.45^{b}	10.13 ± 0.99^{a}	3.98 ± 0.33^{a}	211.12 ± 24.07^{b}	46.34 ± 3.46^{b}
Sawdust + contaminated soil	16.14 ± 1.08^{c}	11.14 ± 0.88^{a}	5.28 ± 0.41^{b}	266.14 ± 21.72^{c}	69.16 ± 2.38^{c}
Poultry litter + contaminated soil	14.57 ± 0.62^{c}	13.70 ± 2.22^{b}	5.17 ± 0.25^{b}	326.70 ± 23.43^{d}	62.38 ± 4.95^{c}
Sawdust + poultry litter + contaminated soil	10.22 ± 0.84^{b}	8.22 ± 1.08^{a}	3.27 ± 0.10^{a}	130.35 ± 20.43^{a}	34.35 ± 3.30^{b}
Banana leaf blades + contaminated soil	1.46 ± 0.70^{d}	1.00 ± 0.48^{c}	0.50 ± 0.24^{c}	28.00 ± 0.00^{e}	2.05 ± 0.00^{d}
Sawdust + NPK + contaminated soil	-	-	-	-	-
Banana leaf blades + NPK + contaminated soil	-	-	-	-	-
NPK + contaminated soil	-	-		-	-

Numbers [a] (means ± standard error of differences) within each column followed by same letters are not significantly different at P≤0.05 (n=6) Duncan's multiple range test.

found out that the substrate composition affected the yield. Other researchers have also reported that cellulosic wastes and poultry litter enhanced the growth of enhanced the growth of fungi in petroleum hydro-carbon contaminated soils (Lamar and Glaser, 1994; Zeddel et al., 1993; Minai-Tehrani and Herfatmanesh, 2007). Schmidt et al. (2008) had also explained that the addition of sawdust or other cellulosic wastes to soil matrix enhances the growth of white rot fungi in soil.

There was no significant difference between spawn and sclerotium in most treatments with regards to stipe and pileus except in contaminated soil substrates, where the sclerotium recorded larger pileus than the spawn (Tables 1 and 2). There was no significant difference between the spawn and sclerotium treatments with regards to stipe height and girth in all treatments applied (Tables 1 and 2). The sclerotium treatment recorded higher yields than the spawn treatment in sawdust + poultry litter + contaminated soil; all other treatments recorded no significant difference between the two inocula (Tables 1 and 2). The fresh and dry weight values show that yields in sclerotium treatments were significantly higher than those in spawn treatments for most treatments except sawdust + poultry litter + contaminated soil and

Table 3. Effect of crude oil contamination on chemical nutrient composition of *Pleurotus tuber-regium* with spawn inoculum.

Substrate composition	Carbohydrate	Protein	Moisture	Fat	Ash	Crude fibre
Control	62.45 ± 0.77[a]	14.07 ± 1.54[a]	12.56 ± 1.23[a]	1.46 ± 0.47[a]	3.39 ± 0.42[a]	12.73 ± 0.21[a]
Contaminated soil only	37.17 ± 3.34[b]	18.60 ± 1.13[b]	6.92 ± 0.42[a]	8.17 ± 0.57[b]	7.46 ± 1.02[b]	16.89 ± 1.15[c]
Poultry litter + contaminated soil	34.77 ± 3.92[b]	20.56 ± 1.27[b]	5.97 ± 0.37[a]	5.93 ± 0.73[b]	6.34 ± 1.28[b]	15.89 ± 2.13[c]
Sawdust + poultry manure + contaminated soil	41.21 ± 1.42[c]	15.65 ± 1.17[a]	8.22 ± 0.60[a]	5.87 ± 0.55[b]	10.28 ± 0.39[b]	11.23 ± 2.12[a]
NPK + contaminated soil	-	-	-	-	-	-
Sawdust + NPK + contaminated soil	58.50 ± 4.80[a]	13.20 ± 1.08[a]	5.80 ± 0.29[a]	1.88 ± 0.46[a]	4.13 ± 0.64[a]	6.97 ± 0.53[b]
Sawdust + contaminated soil	38.76 ± 2.76[b]	26.82 ± 2.14[b]	10.75 ± 0.73[a]	3.94 ± 0.47[b]	9.67 ± 1.13[b]	15.89 ± 1.08[a]
Banana leaf blades + contaminated soil	55.57 ± 5.17[a]	10.92 ± 0.86[d]	6.28 ± 0.66[a]	7.52 ± 0.69[b]	2.69 ± 1.57[a]	12.10 ± 1.52[a]
Banana leaf blades + NPK + contaminated soil	-	-	-	-	-	-

Numbers [a] (means ± standard error of differences) within each column followed by same letters are not significantly different at P ≤ 0.05 (n = 3) Duncan's multiple range test.

Table 4. Effect of crude oil contamination on chemical nutrient composition of Pleurotus tuber-regium with the sclerotium inoculum.

Substrate composition	Carbohydrate	Protein	Moisture	Fat	Ash	Crude fibre
Control	57.62 ± 4.98[a]	12.72 ± 1.07[a]	12.82 ± 1.05[a]	1.65 ± 0.36[a]	4.86 ± 0.48[a]	7.27 ± 1.07[b]
Contaminated soil only	34.40 ± 3.41[b]	21.43 ± 0.44[b]	8.93 ± 0.78[a]	8.81 ± 0.97[b]	11.12 ± 1.11[b]	17.05 ± 0.61[c]
Poultry litter + contaminated soil	34.95 ± 3.42[b]	21.72 ± 1.77[b]	15.52 ± 1.18[a]	2.17 ± 0.53[a]	8.59 ± 0.45[b]	17.05 ± 0.61[c]
Sawdust + poultry manure + contaminated soil	40.83 ± 2.41[c]	18.02 ± 0.61[b]	12.82 ± 1.58[a]	4.96 ± 0.51[b]	13.19 ± 1.03[b]	13.79 ± 1.20[a]
NPK + contaminated soil	-	-	-	-	-	-
Sawdust + NPK + contaminated soil	-	-	-	-	-	-
Sawdust + contaminated soil	42.26 ± 4.12[b]	23.46 ± 0.97[b]	10.91± 1.58[a]	5.02 ± 0.67[b]	10.39 ± 0.75[b]	13.64 ± 0.92[a]
Banana leaf blades + contaminated soil	-	-	-	-	-	-
Banana leaf blades + NPK + contaminated soil	-	-	-	-	-	-

Numbers [a] (means ± standard error of differences) within each column followed by same letters are not significantly different at P ≤ 0.05 (n = 3) Duncan's multiple range test.

sawdust + NPK + contaminated soil treatments (Tables 1 and 2).

Crude oil contamination and the supplementation ion affected the nutrient composition of the mushroom. The substrate composition also affected the quantity of individual nutrient content in the fruit bodies. Crude oil contamination caused a decrease in moisture content although not significantly different from the control that is soils without crude oil contamination (Tables 3 and 4). The ash, fat and protein content of fruit bodies harvested from substrates with sawdust and poultry litter were significantly higher than those harvested from the control and shredded banana leaf blades and NPK treatments (Tables 3 and 4). The carbohydrate contents of fruit bodies from substrates with NPK and shredded banana leaf blades were comparable to that in the control and these were significantly higher than that recorded for the sawdust and poultry litter treatments (Tables 3 and 4). The fibre content in banana leaf blades treatments was however not significantly different from the control and sawdust treatments (Table 3 and 4).

The type of inoculum used affected the nutritive status of mushrooms grown in crude oil contaminated soils. The fat, carbohydrate and crude fibre contents in both sclerotium and spawn grown fruit bodies were not significantly different from each other (Tables 3 and 4). The protein content in sclerotium grown fruit bodies however was only significantly higher than the spawn grown ones in sawdust + poultry litter + contaminated soil substrate (Tables 3 and 4).

The different treatments used affected the nutrient status of *P. tuber-regium* grown in crude oil contaminated soils. The various nutrients varied among the various treatments used and the inocula options. The moisture content varied between 8.93-15.52%, ash between 4.86 - 13.19%, fat between 1.65 - 8.81%, protein between 12.72 - 23.46%, crude fibre 7.27-17.05 and carbohydrate 34.40-52.62 using both sclerotium and spawn values (Tables 3 and 4). This agrees with previous reports that the chemical composition of mushrooms varied based on the type of substrate used and the composition of the substrate (Okhuoya and Ajerio, 1988; Fasidi and Ekuere, 1993; Silva et al., 2002). The protein content of *Pleurotus pulmonarius* Fr. was 29.19% when cultivated on cotton peel. This is comparable to that recorded for *P. tuber-regium* here in the sawdust + contaminated soil substrate combination -26.82% (Table 3). The lipid content was not significantly affected by the substrate composition in the work of Liu et al. (2005). Unlike the current investigation that showed that some substrates significantly affected the fat content of the mushroom (Tables 3 and 4).

The quality of the mushroom fruit body from the crude oil contaminated site was comparable to that cultivated under the best substrate composition for the mushroom. Okhuoya and Ajerio (1988), however recorded a high fat content of 10.20% for *P. tuber-regium* grown in oil palm fruit fibres. The only drawback to the consumption of mushrooms from crude oil contaminated sites is that the sites are always prone to heavy metal contamination. In previous research on this same site, the heavy metal content of fruit bodies when analyzed showed that they accumulated lead, chromium and iron to toxic levels and as such extreme caution should be taken before consumption (Ogbo, 2006).

Conclusion

The study shows that crude oil contamination improves overall well being of the fungus- *P. tuber-regium*. Substrate composition affects the nutrient status of the mushroom grown in crude oil contaminated sites causing variation in the carbohydrate, protein, fat, crude fibre and ash content. Sawdust and poultry litter enhances crude oil fertilizer effect on the fungus while shredded banana leaf blades and NPK fertilizer reduces the fertilizer effect. It is pertinent to consider parameters like heavy metal content before mushrooms from crude oil contaminated sites can be consumed.

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