GROWTH, FRUCTIFICATION OF REISHI (*Ganoderma lucidum)* AND OYSTER (*Pleurotus florida)* MUSHROOMS, AND PHYSICO-CHEMICAL CHANGES OF MEDIUM TREATED WITH DIFFERENT CONCENTRATIONS OF DIESEL

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ABSTRACT

Mushrooms are known to be the most effective degraders of agricultural waste and have been valued as medicinal and food resources. They have unique enzymes that could degrade complex materials, yet little is known for their potential to transform hydrocarbon pollutants into non-toxic compounds. The capability of hydrocarbon utilization was assessed by growing two popular mushroom species of *Ganoderma lucidum* and *Pleurotus florida* in a medium mixed with diesel. Their growth and fructification were assessed in terms of their mycelial growth and fructification parameters such as number of clusters, fruiting bodies, stipe length, cap diameter, and biological efficiency conversion (BEC). The media used in this study were composed of sawdust, rice bran, lime and sugar with a mixture of water and varying concentrations of diesel from 0% to 40%. Mycelial growth of both mushrooms proved to tolerate effectively the diesel concentrations up to 20% but not tolerated in *Ganoderma lucidum* at higher concentration. Fructification occurred in all diesel concentrations for *Pleurotus florid*a, and at 0% and 10% for *Ganoderma lucidum*. The fructification parameters were all affected by added diesel except for stipe length. In terms of media changes, the pH levels showed a decreasing pattern and favored growth and fructification of both mushrooms except for diesel concentrations from 20% to 40% of *Ganoderma lucidum* due to ammonification. Total carbon and potassium of media revealed a decreasing pattern, while nitrogen and phosphorus contents showed an increasing pattern. These patterns of chemical indicators favored mushrooms' capability to grow and tolerate the effect of diesel, considered an environmental pollutant. The effectiveness of both mushrooms in facilitating biodegradation and transformation in varying concentrations of diesel as source of pollutant suggests that they could be employed as bioremediation agents on sites contaminated by hydrocarbon carrying pollutants.

Keywords: mycelial growth, fructification, stipe, ammonification, bioremediation

1 INTRODUCTION

Since the earliest times, mushrooms have been treated as a special kind of food; they have been also considered as the oldest microbial food. In the Philippines, many people in both urban and rural areas are familiar with mushroom-forming fungi growing around them, some of which they exploit for food and medicine.

Caglarlrmak (2007) reported that mushrooms are a good source of vitamins and minerals. Increasing consumption of mushrooms is good for preventing malnutrition. Chang (2007) demonstrated that many mushrooms produce a range of metabolites of intense interest to the pharmaceutical (e.g. anti-tumor, immunomodulation agents, and hypocholesterolaemic agents) and food (e.g. flavor compound) industries. Most mushroom species, if not all, contain biologically active polysaccharides.

Among higher fungi, *Pleurotus* is well acknowledged as an economically important

genus. This may be attributed to its worldwide dispersal, its broad adaptability in growing under various conditions, and, of course its dietetic properties. Thus, *Pleurotus* mushrooms are now cultivated on various agricultural wastes and industrial by-products providing nutritious foods (Zervakis & Balis 1991).

Ganoderma lucidum is a species of *Basidiomycetes* which belongs to *Polyporaceae* (or *Ganodermaceae*) of *Aphyllophorales*. Its fruiting body is called "Reishi" in Japanese and "Lingzhi" in Chinese (Yang & Liau 1998; Wagner et al. 2003). *Ganoderma lucidum,* one of the most famous traditional Chinese medicinal herbs, has been in use as a health food and medicine in the Far East for more than 2000 years already (Fang & Zhong 2002). Lingzhi or Reishi mushrooms contain various chemical substances, including more than 119 different types of triterpenes and several types of polysaccharides (Hsieh & Yang 2004).

Crude oil is a major contaminant of the soil and water in oil producing countries, which is the result of the extraction and processing of oil (Ogbo & Okhuoya 2008). Worldwide concern about the problem of oil spill and its effect has set into motion a scientific inquiry as to how its detrimental effects could be averted or prevented. Many studies on the restoration of sites polluted by harmful chemicals like benzene, toluene, ethylene, and xylene (BTEX) and polycyclic aromatic hydrocarbons (PAH) have shown that it all depends upon the activities of bacteria and fungi, and some plants as well (Isitua & Ibeh 2010).

The concern of this study emanates from the fact that when there is an oil spill in a rural community in the Philippines, irrespective of the volume of the spill, the local farmers usually abandon their farms since these were no longer expected to yield any useful returns. A similar notion is also held in other regions of the world as knowledge of the deleterious effects of petroleum pollution of soils has formed the conclusion that polluted soils are incapable of complete beneficial crop yield and can cause detrimental effect to people's health and the marine environment (Ayotamuno & Kogbara 2007; Oil spill reported in the Philippines 2010, July 6).

White rot fungi like *Ganoderma lucidum* and *Pleurotus florida* are known to be the most effective lignocellulose degraders among the wood degrading microorganisms. They produce unique extracellular enzymes such as lignin peroxidase, manganese peroxidase, laccase and oxidases that generate the hydrogen peroxide needed for peroxidase activity. A diverse group of ligninolytic enzymes has been proven effective in the degradation of polycyclic hydrocarbons, chlorinated aromatic hydrocarbon, polychlorinated biphenyls, the pesticides DDT (dichlorodiphenyltrichloroethane) and lindane, and some azo dyes. The ability of white rot fungi to degrade the abundant naturally occurring polymer lignin has made these fungi appropriate candidates for polycyclic aromatic hydrocarbons (PAH) degradation and other constituents present in the crude oil (Emuh 2010).

In this study, edible white rot fungi that belong to the class *Basidiomycetes* such as *Pleurotus florida* were used since some *Pleurotus* species are known for their ability to degrade hydrocarbon containing compounds (Sarkanen et. al. 1991). Furthermore, *G. lucidum,* whose medicinal properties have been explored (Lin et. al. 1995; Park et. al. 1997), was studied further in comparison with *Pleurotus florida* for its ligninolytic system and biodegradation potential, of which little is presently known. The aim of the study was to compare both the mushrooms' capability to tolerate the effect of varying diesel concentrations in terms of mycelial growth, fructification and analysis of media pH, C, and NPK contents to determine whether or not they are good bioremediation agents against pollutants.

2 MATERIALS AND METHODS

2.1 Grain Spawn Preparation

Spawns of *Ganoderma lucidum* and *Pleurotus florida* were reproduced using sorghum grains. The procedures used for preparation of spawn were adopted from Ogbo and Okhuoya (2008). The inoculated bottles of sorghum were incubated at room temperature, about 25-27 ^{O}C for 14 days or until the full ramification of the spawn substrate. The fully grown spawn cultures were used for the next procedure of inoculating the fruiting bag.

2.2 Fruiting Bags Preparation

As adopted from Oei and Nieuwenhuijzen (2005), there were four main stages of fruiting bag preparation as enumerated below:

1. **Dry substrate preparation.** Prior to the mixing of the liquid part (moisture content) of the substrate, a dry mixture of composted sawdust (78%), rice bran (20%), brown sugar (1%), and lime (1%) was used as substrate for growing mushroom mycelia of *G. lucidum* and *P. florida.* All the ingredients were mixed thoroughly until the mixture had become homogenous to ensure even distribution of all nutrients for the mushroom growth. The mixture was divided into five equal portions to accommodate five treatments for each mushroom species. Proper moisture content was set at field capacity level of the substrate, about 60-65 percent (Oei & Nieuwenhuijzen 2005). The moisture portion of the substrate was composed of the various concentrations of crude oil (10%, 20%, 30%, and 40%) and tap water as control. This level of concentration was adopted from Adedokun and Ataga (2006). To make 100 bags of substrate for both *G. lucidum* and *P. florida*, 40 kg. of dry substrate (31.2 kg sawdust, 8.0kg rice bran, 0.4 kg brown sugar, 0.4 kg lime) and 24 L of liquid portion (19.2 liters tap water and 4.8 liters diesel) were used, as indicated in Table 1.
2. **Crude oil and substrate mixing procedure.** The dry substrate composed of 40 kg was divided into five equal parts, yielding 8 kg for each part. The liquid part of the mixture at 60% moisture content was composed of 22 L or 4.8 L each, if divided into five equal parts. From this 4.8 L. value, the various concentrations of crude oil were obtained. Table 1 shows the varying amounts of diesel and water needed for different concentrations in each treatment. The remaining parameters had the same values such as dry substrate mass, total mass and approximate yield for a 600-gram substrate bag with a yield of 21 600-g bags. This was enough for ten replicates on each treatment for both mushrooms. After both the substrate and the various concentrations of crude oil had been measured, they were then mixed thoroughly.

Table 1: Fruiting bag ingredients formulation at different concentrations of diesel.

Percent Diesel	Dry Substrate	Crude Oil (Diesel)	Water	Total Mass	Yield (600-g/bag)
(Control)	8 Kg	0	4.8 L	12.8 Kg	21
10%	8 Kg	0.48 L	4.32 L	12.8 Kg	21
20%	8 Kg	0.96 L	3.84 L	12.8 Kg	21
30%	8 Kg	1.44 L	3.36 L	12.8 Kg	21
40%	8 Kg	1.92 L	2.88 L	12.8 Kg	21

1. **Bagging and sterilization of substrate.** The same treated substrate groupings (0%, 10%, 20%, 30%, 40%) were used for both *G. lucidum* and *P. florida* mycelia. Prior to sterilization of substrate, 5"×10" heat-resistant polypropylene plastic bags were used. Each bag was composed of 600 g of substrate, with 10 replicates for each treatment. There were a total of 100 bags for both *G. lucidum* and *P. florida*, or

a total of 60 kilograms of substrate. After placing the substrate in the bag, a one-inch PVC ring was placed around each bag, thereby providing the neck. Each bag was plugged with cotton, covered with paper, tied with rubber band, and labelled. The bags were then autoclaved for one hour at 15 psi pressure as recommended by Stamets (1993).

2. **Inoculation and Incubation for Mycelial Colonization of the Fruiting Bags.** After sterilization, the bags were transferred to the inoculation room and allowed to cool. Upon cooling of the bags, pure spawn cultures were inoculated aseptically. This was done by cleaning the inoculation table with bleach (Sodium hypochlorite, 5.25% by weight) prior to the opening of the sterilized substrate bags and grain spawns bottles. An alcohol lamp was lighted to sterilize a thin iron rod used to probe and pour the grain spawn from the 350-ml catsup bottle to the spawn bag opening. At the same time, the lighted alcohol lamp ensured the keeping of undesirable air-borne microorganisms from penetrating both the grain spawn bottles and the substrate bags upon opening and closing during the inoculation process. After inoculation of all the spawn bags was accomplished, the inoculated bags were incubated at room temperature without exposure to much light for 28 days, or until mycelium had fully ramified (Akyuz & Yildiz 2008). Mycelial growth rates were observed at this stage of the experiment.

2.3 Fruiting Room Maintenance

The maintenance of fruiting room for mushrooms was carried out according to specifications by Quimio (2002). After the mycelia of *G. lucidum* and *P. florida* had fully colonized the fruiting bags, the bags were then transferred to the fruiting room and opened to trigger fructification. Daily watering of about 2-4 times a day (depending on the prevailing weather condition) was consistently done to maintain the desired temperature and increase humidity. Pouring of lime (CaO) on the ground was also done on a weekly basis to prevent growth of green molds. Fruiting bags contaminated with green molds were removed to prevent contamination of the other bags. Continuous lighting was also done using 20 watt daylight fluorescent tube. Door was kept closed and net screens intact to avoid infestation from flies.

2.4 Data Collection

Both mushrooms had ten replicates per treatment. To help assess the growth of mushrooms in this experiment, eight parameters were used, namely: mean mycelial growth rate, pH and essential minerals (C, N, P, and K) of media, cap diameter, number of clusters, number of fruiting bodies, stipe length, and biological efficiency conversion (BEC). All data were collected and recorded using Microsoft Excel and fed into the SPSS program for ANOVA and *t* test. Chemical analysis of media was done at day 0 and at the end of flushing (fruiting), 8 weeks after inoculation.

Biological Efficiency Conversion. To determine the Biological Efficiency Conversion, harvesting of mature mushroom fruiting bodies was done on a daily basis. The fresh weight of the mushrooms harvested per flush from each bag for each treatment was taken and recorded until the fourth week of flushing.

The efficiency of utilization of the substrate by the mushroom mycelia was calculated using the formula reported by Stamets (1993) as follows:

$$\text{Biological Efficiency Conversion (BEC)} = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the substrate used}} \times 100$$

3 RESULTS AND DISCUSSION

Both white rot fungi species of *Ganoderma lucidum* and *Pleurotus florida* were able to hydrolyze diesel fuel molecules without affecting mycelial growth at 10% level of concentration, having placed or passed the range of 120 millimeters which was the same with the control (0% diesel concentration). The key level of diesel concentration observed was at 20%, where the mean mycelial growth of *G. lucidum* dropped below the range of 120 millimeters and up, while the mycelia of *P. florida* remained the same. At 30% and 40% level of diesel concentration, the same trend of decreasing growth rate for *G. lucidum* was observed (Figure 1).

Figure 1: Appearance of spawn bags during mycelial growth stage of *Ganoderma lucidum* and *Pleurotus florida* at 0% - 40% diesel concentration

This finding suggests that *G. lucidum* has less potential in biodegradation of diesel fuel pollutant compared to *P. florida* when the level of pollutant concentration is higher than 20% (Figure 2) The outstanding mycelial growth of *P. florida* in all concentrations may be due to higher production of extracellular enzymes that enabled it to utilize the hydrocarbons faster. This result agreed with the findings of Stamets (2005) that *P. florida* produces extracellular enzymes such as lignin and cellulose peroxidases that hydrolyze long chains of hydrocarbons like petroleum products and pesticides. The inhibited growth of *G. lucidum* might be due to the presence of diesel fuel additives such as sulfur, amines, phenols and benzene (Odjegba & Sadiq 2002).

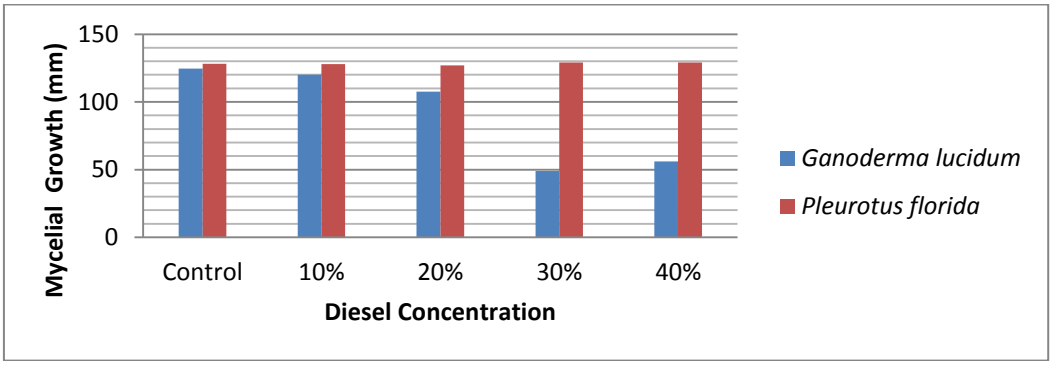

Figure 2: Comparative mycelial growth rates of *Ganoderma lucidum* and *Pleurotus florida* after 28 days (Week 4) of incubation given different concentrations of diesel in the substrate

The *t* test was employed on each concentration level on a weekly basis. There was no significant difference from Week 1, Week 2 and Week 3. However, at the end of Week 4, there was a significant difference at concentrations of 30% (*GL*, *M* = 49.0000, *SD* = 37.25289; *PF*, M = 129.0000, SD = 3.16228 *p* = .003) and 40% (GL, *M* = 56.0000, *SD* = 33.56586; PF, *M* = 129.0000, *SD* = 3.732328, *p* = .002) (Table 2).

Table 2: Comparative effect of diesel treated substrate at different concentrations on mycelial growth of *Ganoderma lucidum* and *Pleurotus florida* at week 4

Treatment	Mean (*M*)	Standard Deviation (*SD*)	Treatment	Mean (*M*)	Standard Deviation (*SD*)	Sig.
GL-0%	124.50	5.99	PF-0%	128.10	3.41	.120
GL-10%	120.00	9.13	PF-10%	128.00	4.22	.058
GL-20%	107.60	17.85	PF-20%	127.00	4.83	.131
GL-30%	49.00	37.25	PF-30%	129.00	3.16	.003
GL-40%	56.00	33.57	PF-40%	129.00	3.73	.002

These data show that in terms of effectiveness on biodegradation or capability to grow on substrate treated with diesel, there was no significant difference between *G. lucidum* and *P. florida* up to 20% level of diesel concentration. At concentrations of 30% and 40%, *P. florida* was found to be more effective in biodegradation of diesel treated substrates. One of the factors observed in the mycelial growth of *G. lucidum* was its thickness. A decrease in thickness was observed when the concentration of diesel reached 30% and 40%. *G. lucidum* showed a lower rate of mechanical penetration and breaking down of substrate compared to *P. florida*. This is an indication that mycelia of *Ganoderma* showed a different growth behavior when it comes to biodegradation of diesel crude oil. This may be attributed to diesel additives as reported earlier (Odjegba & Sadiq 2002; Adedokun & Ataga 2006). Another factor might be the nutrient supply of the substrate that was suitable for *Pleurotus* species but not for *Ganoderma.* This finding agrees with the findings of Sang-Hwan et al. (2007) and Thiemann and Palladino (2009) that proper nutrient supply must be incorporated to attain enzyme-substrate balance. Both mushrooms could be effective in degrading pollutants provided that proper and appropriate nutrient supplies were met. In that way fungus can regenerate and produce enzymes necessary for biodegradation of crude oil and other types of pollutants (Travisano & Velicer 2004).

Fruiting bodies were not formed in some concentrations of *Ganoderma lucidum*. Primordial emergence was only observed at 0% and 10% diesel concentrations, 28 days after opening the bag for fruiting. No fruiting bodies were formed starting from 20% through 40%, although at this level, mycelial growth tolerated the level of diesel concentration (Figure 3). Lack of mycelial thickness was the main cause of inhibited fructification. This agreed with the report of Hudson (1986) that dense and regular branching of hyphae endows fungi with potentials to pervade any substrate thoroughly. In other words, the higher the mycelium thickness, the higher the rate of mechanical penetration and breaking down of substrate. Fruiting bodies were formed in all concentrations of *Pleurotus florida* but not all manifested the same prolific emergence as shown in Figures 3. Delayed fruiting emergence was also observed in concentrations of 30% and 40%.

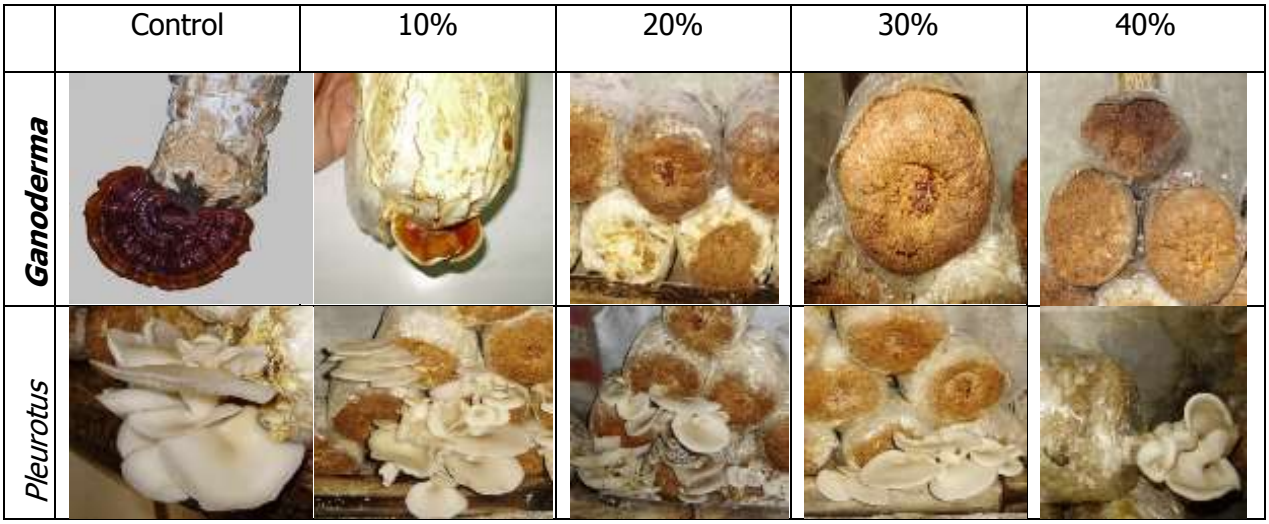

Figure 3: Appearance of spawn bags during fructification stage of *Ganoderma lucidum* and *Pleurotus florida* at 0% to 40% diesel concentrations

Some abnormalities in shape and color were also manifested at 40% diesel concentration. There was discoloration and deformity in shape or abnormal growth as compared to the normal fruiting bodies (Figure 4). This condition showed that *P. florida* exhibited tolerance as the diesel concentration of the substrate increased.

Figure 4: Appearance of some abnormalities on fruiting bodies of *Pleurotus florida*

The increase in diesel concentration (pollutant) possibly caused the mushroom's decreased enzyme activity as it could not balance with the amount of hydrocarbons in the substrate (Stoker 2011). This agreed with the report of Tokimoto et al. (1987) that decrease in enzyme activity could lead to failure of cell wall formation, thus resulting to discoloration and malformed shapes of mushroom fruiting bodies. This might be attributed also to the decrease in pH level (Stamets 2000).

Except for stipe length, all the parameters of fructification were significantly affected by the increase of diesel concentration in the substrate (F = .411, p = .800). Statistical analysis showed that the number of clusters ((F = 24.70, p = .001), number of fruiting bodies (F = 30.89, p = .001), cap diameter (F = 2.61, p = .048), and biological efficiency conversion (F = 22.90, p = .001) were significantly different with the change in diesel

concentration. These data show that the mean values were affected inversely proportional; as the diesel concentration increased, the values of fructification parameters decreased (Figure 4).

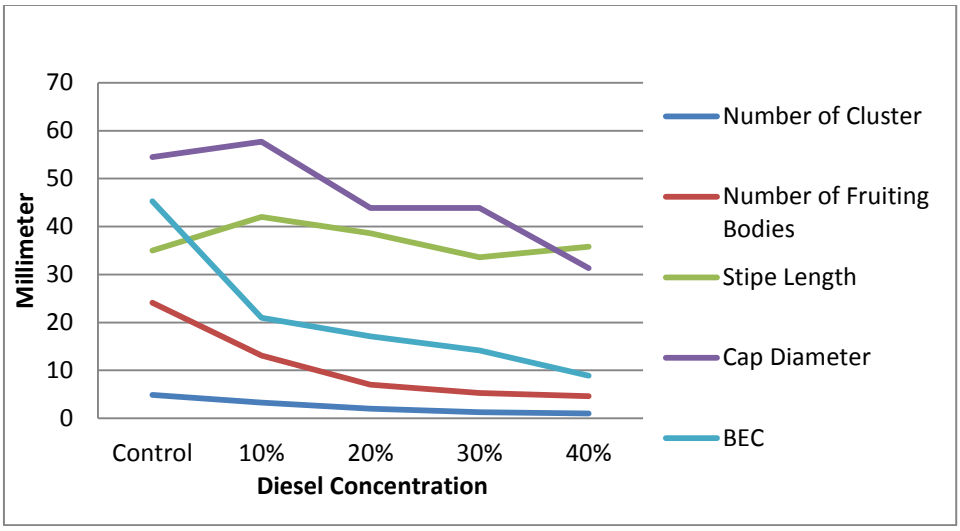

Figure 4: Average fructification parameters of *Pleurotus florida* in terms of numbers of clusters and fruiting bodies, lengths of stipe and cap diameter and biological efficiency conversion (BEC)

This result agrees with the findings of Ogbo and Okhouya (2008) that some *Pleurotus* species revealed significant changes in biodegradation of crude oil. Likewise, diminished growth due to the presence of pollutants was also observed. Stipe length, on the other hand, showed insignificant difference with the increase in diesel concentration. This might be attributed to some sort of growth abnormalities like deformed fruiting bodies and longer than normal stipe length. It is, therefore, expected that stipe length might not show significant difference with the increase in diesel concentration as observed in earlier studies (Tokimoto 1987; Stamets 2000; Hamman 2004).

3.1 Overall Discussion

Both mushrooms were able to utilize and tolerate the addition of crude oil in the media to as high as 40% concentration. Mycelial growth confirmed this claim, although *Ganoderma lucidum* exhibited thinner growth compared to *Pleurotus florida* at higher concentrations of 30% and 40%. Mycelial growth and fructification are indicators of the ability of mushrooms to initiate biodegradation of diesel hydrocarbons added in the media. Nutrients in the media provided are sources of carbon, nitrogen, phosphorus, and potassium. These basic essential nutrients are important for mushroom health and activity while biodegradation of foreign materials like diesel takes place. In this way, the nutrients provided for mushrooms would ensure the production of extracellular enzymes for the degradation of diesel. Degraded hydrocarbons of diesel were assumed to be converted by mushrooms into non-toxic compounds of water, carbon dioxide, and adenosine triphosphate (ATP). These compounds are important in plant growth and some other biotic components of the environment. This natural phenomenon indicates that mushrooms not only provide food but also serve as transformers of environmental pollutants into non-reacting organic compounds, like some other microorganisms do.

The media mixed with different concentrations of diesel exhibited some changes after the inoculation of mushroom spores. The pH level normally changed from neutral to

acidic due to organic acid release (Table 3). Extracellular enzymes of mushrooms made the media acidic after degradation took place. Alkalinity of media indicates that biodegradation of media was not completed and possible ammonification had taken place due to the invasion of other ammonia-producing microorganisms like bacteria (Stamets 2000; Stoker 2011).

In terms of media nutrient changes, as shown in Table 3, carbon (C) and potassium (K) exhibited a lowering percentage levels after the introduction of mushrooms spores. The lowering nutrient levels of C and N is a normal phenomenon for biodegradation activity. Carbon compounds coming from the media and added diesel hydrocarbons were utilized by the cells and transformed into carbon dioxide gas and released in the atmosphere. In this manner, lowering amount of C was observed in the media. Potassium on the other hand had almost the same trend. After mushroom mycelia utilized K for osmotic regulation of cells, protein metabolism, and some other enzyme activities, the amount of K changed into a limiting level. This indicates biodegradation activity of diesel hydrocarbons. The lowering level of K, unlike carbon that is released in the atmosphere, might be attributed to leaching due to its solubility in water. This happened during the watering of mushrooms on a daily basis during the fruiting stage (Ayotamuno & Kogbara 2007).

Table 3: Percent increase and decrease (-) of media conditions in fruiting bags of *Ganoderma lucidum* and *Pleurotus florida* 56 days after inoculation

Media	Control		10%		20%		30%		40%	
Condition	GL	PF	GL	PF	GL	PF	GL	PF	GL	PF
pH	(-)29	(-)27	(-)37	(-)34	21	(-)24	21	(-)24	13	(-)31
Carbon	(-)24	(-)11	(-)33	(-)22	(-)25	(-)44	(-)25	9	(-)15	(-)0.3
Nitrogen	5	6	33	37	93	27	81	33	46	56
Phosphorus	1100	800	471	586	383	533	271	457	350	412
Potassium	5	(-)18	(-)8	(-)29	(-)8	(-)13	(-)23	(-)14	71	(-)13

Another consideration in media changes is the increasing amount of nitrogen (N) and phosphorus (P). These two elements unlike K are structural components of the cell and therefore remain or provide N-P supply after composting or biodegradation has taken place. In this accord, their percentage levels are expected to be higher after the inoculation of mushroom spores in the media. However, it is important to note that initial amount of these two elements prior to the introduction of mushroom spores to the media should at least obey the law of conservation of mass. This means that initial amount should always be equal to the final amount. This further means that the increased percentage levels of N-P after the introduction of mushroom spores to the media could not be attributed to mushrooms alone. Noteworthy is the fact that mushrooms are only utilizing N-P, not creating them out of nothing. In the case of N, the increased percentage values could be attributed to the initial N in the media and some other sources, particularly the atmospheric nitrogen (N_2). In this manner, it could be said that mushrooms are capable of fixing N_2 in the air. In the case of increased level of P in the media, noteworthy is the fact that there is no other source for P except the media itself, unlike N that is readily available from the atmosphere. Water analysis revealed only 0.01 to 0.04 ppm (parts per million) level of P, very low and insignificant source to make P level increase to more than 1000%. Daily watering of mushrooms with so little ppm levels of P would not be attributed to such huge increase in the percentage values of P in the media. At mineral level, DNA replication does not create P in the formation of phosphate group without having the initial source. In this manner, DNA does not create but only utilize the P available in the media or immediate environment. The increase in P level in the media is somewhat attributed to the possible ability of mushrooms

to facilitate biological transmutation, a transformation of one chemical element into another element by means of nuclear reactions (Kervan 1998). In this theory, sources of P are the fusions of the following nuclear reactions:

$Mg^{24} + Li^{7} \rightarrow P^{31}$
$2O^{16} - H^{1} \rightarrow P^{31}$
$N + O \rightarrow P$

According to Biberian (2012) this phenomenon of possible transmutation occurrences, though not understood fully, still reveals undeniable facts that need scientific explanation.

4 CONCLUSION

The effectiveness of both mushroom species of *Ganoderma lucidum* and *Pleurotus florida* in facilitating biodegradation and transformation was assessed using the parameters of mycelial growth, fructification, and media changes given different concentrations of diesel. Both mushrooms were effective in utilizing and tolerating the effect of the added diesel in the media at varying concentrations as high as 20%. *Pleurotus florida* showed a higher tolerance level then *G. lucidum* at concentrations up to 40%.

The effectiveness of both mushrooms in facilitating biodegradation and transformation in varying concentrations of diesel as source of pollutant suggests that they could be employed as bioremediation agents on sites contaminated by hydrocarbon carrying pollutants.

5 RECOMMENDATIONS

Studying the mechanism of biodegradation of crude oil is a young technology that needs to be further explored. This could lead to commercialization of bio-remediating agents like white rot fungi. In the light of these findings the study recommends that:

- Further studies are needed to determine biodegradation parameters like at what concentration a certain species of fungus can tolerate certain pollutants like hydrocarbon fuels and DDT.
- It is also recommended that studies be done to increase the biodegradation rate of potential fungal microorganisms like *G. lucidum* and *P. florida* by determining the specific enzymes produced by each of these mushrooms.
- More parameters like gasoline, used engine oil, and pesticide might be employed to check which mushroom would best fit the condition. In this manner, the significant difference in comparing different varieties of mushrooms might be greater.
- Analysis of heavy metals in the fruiting bodies might be another way of testing the effectiveness of both mushrooms not only to utilize the chains of hydrocarbons but also to bio-accumulate some aggregates of petroleum particulates.
- For education purposes and enhancement of environmental awareness among students, mushroom experiments could be employed in the laboratory class procedures, but this requires more time and larger facility if each and every procedure of mushroom cultivation will be followed religiously. It might be best to just procure spawn bags or ready-made mushroom mycelia available from some colleges and universities with microbiology laboratory facilities. With dedicated and enthusiastic teachers, there will be no hassle in making this tedious laboratory work in the class.

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