Mycoremediation of Pesticide Contaminated Soil Using Mushroom *Pleurotus ostreatus*

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Abstract

The indiscriminate use of the Sniper Branded pesticide (2,2-dichlorovinyl dimethyl phosphate (DDVP) containing pesticide) in recent times in the country and its reported persistence in the environment has made it an environmental and health concern for the country. This study investigated the ability of *Pleurotus ostreatus* in degrading the stated DDVP containing pesticide substance in contaminated soil samples under laboratory controlled environment. The experimental setup include soil samples contaminated with three concentrations of the pesticide: 10% v/w, 30% v/w and 60% v/w, the soil samples were then inoculated with vigorously growing spawns of *P. ostreatus.* The setup was then incubated for 90 days, after which the pesticide concentration, nutrient content, organic content, pH, and heavy metal content of the soil samples were determined. The results of the study showed that the nutrient and organic content of the soil decreased after 90 days' incubation with mushroom. Furthermore, the heavy metal contents of the soil samples incubated with mushroom had significantly ($p<0.05$) lower proportions of heavy metal content. Of note is the total clean-up of the lead ion concentration of the pesticide polluted soil for all concentrations prepared in the study. Additionally, incubation with *P. ostreatus* significantly ($p<0.05$) reduced the pesticide concentration in the soil by 57.74% for the 10% concentration and by 31.64% for 60% concentration. The results showed that *P. ostreatus* is useful in the bioremediation of soils contaminated with pesticides containing DDVP on a small scale project. Therefore, the study recommended that soil contaminated with the DDVP pesticides may be treated with *P. ostreatus* to reduce heavy metal contents and total pesticide concentration in the soil to the generally acceptable levels that may not be deleterious to the environment and human health.

Keywords: Human Health, Soil, Pesticide, Nutrient, Organic content, Food, Agriculture

Introduction

The Food and Agriculture Organization (FAO) has defined pesticide as: any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies (FAO, 2002). There have been no doubts about the importance of pesticides in maintaining a vector and disease free environment in the modern day environment. On the other hand, due to their widespread use, pesticides are currently detected in various environmental matrices such as soil, water and air and there is great concern about their potential environmental hazard (Ahemad *et al.,* 2009). Contamination with pesticides can lead to pollution of the soil, surface and groundwater, reduced biodiversity and depression in soil heterotrophic bacteria (including denitrifying bacteria), and fungi (Uqab *et al.*, 2016).

Their targeted inherent toxicity aside, pesticides can sometimes migrate along the food chain to extend their toxicity to other 'non-target' organisms within the ecosystem. Some pesticide compounds have been known to biomagnify along the food chain before eventually reaching the human food sources, an example of this is the DDVP (2,2dichlorovinyl dimethyl phosphate) pesticide family. Some studies have found premises to link DDVPs to some cancers in the population (Agrawal and Sharma, 2010).

The Sniper branded pesticide (sold in Nigeria) contains chemical compounds belonging to the DDVP pesticide family (Kemabonta and Amadi, 2014). Thus its indiscriminate use of this pesticide in recent times in the Nigeria might be building into an environmental nuisance for the country at large (Kemabonta and Amadi, 2014). Since the environmental and health consequences of DDVPs are well reported in literature, there is need to find an easy, cheap and effective method to successfully curb the advent of this emerging environmental trend. Various methods of cleaning up the persistence of DDVP and other pesticides in the environment have been suggested in literature (Gavrilescu, 2005) including the use of bacteria consortia and some phytoremediation techniques (Gavrilescu, 2005). However, these techniques require highly skilled and specialized expertise that is not readily available in the environment especially in developing countries.

An alternative to these methods remediating pesticide pollutants is mycoremediation. Mushrooms: which are the major material needed for mycoremediation, are readily available at cheaper rates in the environment. Though the most common pesticide compounds are moderately to very persistent in the environment, mushrooms have been said to possess the ability to degrade them (Uqab *et al.*, 2016). Whether, *P. ostreatus* has the abilities to remediate DDVP contaminated soils is still uncertain. The aim of this study is to investigate the ability of *P. ostreatus* in remediating DDVP contaminated soils. at different levels of concentration.

Materials and Methods

Source of Materials

Top soil sample used for this experiment was collected within 1 to 10 cm depth from the nursery site of the Botanical garden of the University of Lagos, Akoka which is situated in the North-East of Yaba, Lagos State, Nigeria. It lies in the latitude $6^0$31'0"N and longitude $3^0$23'10"E. Pure culture of *Pleurotus ostreatus* was collected from a commercial mushroom laboratory of Federal Institute of Industrial Research Oshodi (F.I.I.R.O.) Lagos Nigeria. The sniper pesticide products used for the study were purchased from Mile 12 market, Lagos, Nigeria.

Contaminant Composition

Three concentrations of pesticides were prepared for the study, namely 10% v/w, 30% v/w and 60% v/w. The 10% v/w was prepared by weighing 400g of soil sample, this weight was mixed with 40 ml of sniper pesticide, while the 30% v/w was prepared by weighing 400g of soil sample, this weight was mixed with 120 ml of sniper pesticide. Finally, the 60% v/w was prepared by weighing 400g of soil sample, this weight was mixed with 240 ml of sniper pesticide.

Experimental Set Up

Fungal spawns collected from the Biotechnology Department of the Federal Institute of Industrial Research, Oshodi were multiplied based on a modified method adapted from Fasidi *et al.,* (2008). The experimental set up adopted a modified method of Adenipekun and Fasidi (2005). The prepared soil concentrations were mixed thoroughly with 40g of saw dust, 40g rice bran and 10g of calcium carbonate. The entire contents were then pasteurized inside the pasteurizing drum. After cooling, each pot was inoculated with 5g of growing spawns of the fungus in an inoculating chamber. The inoculated pots were then arranged on shelves inside the inoculating room for 90 days. However, the control samples were not contaminated with any of the mixture of contaminants and they were not inoculated with the fungus. At the end of the incubation period the soil samples from each pot were analysed for their physiochemical and pesticide concentration parameters. This experiment was carried out at the mushroom house at the Federal Institute of Research, Oshodi.

Analysis of the Total Pesticide Content

The amount of pesticide in the soil sample was measured before and after the remediation process using a modified gas chromatography method described by Chen *et al*., 2011.

Analysis of the Soil pH and Nutrient content

Soil pH was determined using pH meter 3015 (Jenway, U.K.). The organic and nutrient contents of the soil was determined using modified methods adapted from Methods of Analysis, Association of Official Analytical Chemists (AOAC), 2003

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Soil Heavy Metal Content Analysis

The soil samples were digested using a modified method adapted from Ritter *et al*., 2004. While the heavy metal concentrations were determined using the spectrometry method of Atomic Absorption Spectrometer (AAS) with a hollow cathode lamp and a fuel rich flame (air-acetylene).

Results

Soil pH, Nutrient and Organic Content

The soil sample with the lowest nitrogen content was the uncontaminated (0%) soil standing as 1.88 mg/kg. while the soil sample with the highest nitrogen content was the soil containing 60% pesticide, with a value of 6.71 mg/kg (**Table 1**). This same trend is evident in the phosphorus, sulphur and nitrate content of the soil sample. In all the above, the uncontaminated (0%) soil had the least value while the most contaminated (60%) had the highest value. On the other hand, the observed sulphate and potassium contents showed that the uncontaminated soil had the highest sulphate and potassium content, while the most contaminated (60%) soil sample had the lowest sulphate and potassium content. The nitrogen content of the soil samples decreased after 90 days (**Table 2**). The largest decrease in nitrogen content was seen in the soil sample that had the largest level of contamination (60%), while the least decrease was observed in the soil sample contaminated with 10% pesticide solution. This same trend of decrease values was observed for the Sulphate, potassium phosphorus and sulphur content of the soil. However, the nitrate contents of the soil seem to increase after 90 days. The soil sample with the lowest organic carbon content was the uncontaminated (0%) soil standing as 15.36 mg/kg (**Table 2**). while the soil sample with the largest organic carbon content was the soil containing 60% pesticide, with a value of 32.20 mg/kg. The same can be said for the organic matter and moisture content of the soil sample. Additionally, the moisture contents and pH of the soil seem to increase after 90 days.

Table 1: Nutrient Content of the Soil

Concentration		Nitrogen (mg/kg)	Sulphate (mg/kg)	Potassium (mg/kg)	Phosphorus (mg/kg)	Sulphur (mg/kg)	Nitrate (mg/kg)
0%	Initial	1.88 ±0.04^{a}	72.75±0.19^{a}	20.89 ±0.08^{a}	20.30 ±0.17^{a}	6.61 ±0.17^{a}	10.55±0.17^{a}
	W/out Mush	1.52 ±0.01^{a}	70.15 ±0.19^{a}	19.4±0.36^{a}	19.28±0.17^{a}	4.77±0.23^{b}	11.73±0.29^{b}
	With Mush	0.95±0.04^{ab}	54.94±0.62^{b}	13.4±0.36^{c}	16.24±0.22^{b}	2.51±0.17^{bc}	6.3±0.12^{c}
	% change due to mush	37.50%	21.68%	30.93%	15.77%	47.38%	46.29%
10%	Initial	2.36±0.03^{a}	68.25±0.14^{a}	19.7±0.17^{a}	23.03±0.13^{b}	9.26±0.04^{a}	14.03±0.11^{a}
	W/out Mush	2.28±0.15^{a}	67.46±0.09^{a}	11.2±0.11^{b}	29.59±0.08^{a}	6.55±0.13^{b}	14.75±0.06^{a}
	With Mush	1.96±0.03^{a}	51.85±0.14^{b}	7.1±0.08^{c}	17.54±0.13^{c}	4.41±0.10^{bc}	7.46±0.17^{b}
	% change due to mush	14.04%	23.14%	36.61%	40.72%	32.67%	49.42%
30%	Initial	3.81±0.04^{a}	60.61±0.16^{a}	17.57±0.12^{a}	26.44±0.12^{a}	11.67±0.12^{a}	16.35±0.11^{a}
	W/out Mush	2.76±0.09^{b}	61.23±0.26^{a}	8.00±0.18^{b}	17.42±0.21^{b}	7.97±0.19^{b}	16.97±0.06^{a}
	With Mush	2..6±0.1^{bc}	43.15±0.12^{b}	4.87±0.1^{c}	13.77±0.12^{c}	6.01±0.15^{bc}	9.72±0.11^{b}
	% change due to mush	5.80%	29.53%	39.13%	20.95%	24.59%	42.72%
60%	Initial	6.71±0.09^{a}	64.45±0.07^{a}	13.69±0.03^{a}	34.44±0.24^{a}	18.24±0.21^{a}	18.63±0.07^{a}
	W/out Mush	3.59±0.01^{b}	53.55±0.04^{b}	4.65±0.04^{b}	22.42±0.25^{b}	11.41±0.12^{b}	19.34±0.11^{a}
	With Mush	2.45±0.11^{bc}	33.06±0.08^{c}	3.88±0.08^{bc}	18.29±0.08^{c}	8.29±0.075^{c}	6.3±0.11^{b}
	% change due to mush	31.75%	38.26%	16.56%	18.42%	27.34%	67.43%

Values in the same column followed by different letter are significantly different according to Duncan Multiple Range test ($P<0.05$)

Table 2: Organic Content and pH the Soil

Concentration		Organic Carbon Content (%)	Organic Matter (%)	Moisture (%)	Soil pH
0%	Initial	15.36±0.04 [a]	26.54±0.1 [a]	30.74±0.18[c]	6.72±0.01[a]
	W/out Mush	8.69±0.02 [b]	14.58±0.26 [b]	39.37±0.28 [a]	6.91±0.02[a]
	With Mush	4.32±0.21 [c]	8.86±0.08 [c]	35.7±0.16 [b]	7.06±0.05[b]
	% change due to mush	50.29%	39.23%	9.32%	2.17%
10%	Initial	17.65±0.31 [a]	30.92±0.14 [a]	36.06±0.19 [c]	6.96±0.01 [a]
	W/out Mush	9.34±0.23 [b]	16.35±0.21[b]	45.73±0.06 [a]	7.11±0.01 [a]
	With Mush	5.80±0.03 [c]	9.99±0.1 [c]	41.39±0.06 [b]	7.06±0.0 [a]
	% change due to mush	40.74%	43.62%	11.02%	0.72%
30%	Initial	19.68±0.23 [a]	34.29±0.13[a]	49.78±0.11[c]	6.64±0.01 [a]
	W/out Mush	12.87±0.17 [b]	22.26±0.28[b]	59.59±0.35 [a]	6.79±0.0 [a]
	With Mush	8.23±0.11 [c]	14.22±0.19[c]	54.75±0.1[b]	6.81±0.01 [a]
	% change due to mush	36.05%	36.12%	8.12%	0.29%
60%	Initial	32.20±0.05 [a]	55.71±0.03[a]	64.91±0.01[c]	6.50±0.02 [a]
	W/out Mush	19.56±0.16 [b]	33.58±0.04 [b]	68.78±0.1[a]	6.69±0.01 [a]
	With Mush	12.7±0.17 [c]	21.79±0.13[c]	65.65±0.1[b]	6.77±0.01 [a]
	% change due to mush	35.07%	35.11%	4.55%	1.20%

Values in the same column followed by different letter are significantly different according to Duncan Multiple Range test ($P<0.05$)

Heavy Metal Content of the Soil

The Lead content of the soil generally increased with increased contamination with the sniper pesticide (**table 3**). The soil sample with the lowest lead content was the uncontaminated soil (0%) standing as 0.0215 mg/kg. while the soil sample with the largest lead content was the soil containing 60% pesticide, with a value of 0.089 mg/kg. This same trend of proportional increase with increased contamination is evident in the Zinc and Manganese content of the soil sample. On the other hand, the trend observed in the copper contents was inversely proportional to increased contamination. The uncontaminated soil had the highest copper content, while the most contaminated (60%) soil sample had the lowest copper content. However, the iron content of the soil was not associated with the level of contamination in each soil sample. The Iron content of the soil samples decreased after 90 days (**table 4**). The highest decrease in iron mineral content was seen in the soil sample that had the largest level of contamination (60%), while the least decrease was observed in the soil sample contaminated with 30% pesticide solution.

The iron content of the soil samples of the samples that were incubated without mushroom were significantly higher ($p<0.05$) than those that were incubated with mushrooms. The largest difference was observed in the soil samples without any form of contamination (0%). The same trend was also observed for zinc and manganese content the soil samples. However, the differences in the lead contents of soil incubated with and without mushroom were not significant. The same can be said of copper were the only significant difference occurred in the control setup.

Table 3: Heavy Metal Content of the Soil

Concentration		Iron (mg/kg)	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)	Manganese (mg/kg)
0%	Initial	49.45±0.03 [a]	0.022±0.0[a]	2.71±0.06 [a]	15.94±0.02 [a]	10.70±0.04 [a]
	W/out Mush	18.04±0.03 [b]	0.012±0.0 [b]	1.22±0.01 [b]	10.0±0.13 [b]	9.35±0.23 [b]
	With Mush	10.43±0.11 [c]	0.001±0.0[c]	0.2±0.01 [c]	4.93±0.19 [c]	4.27±0.06 [c]
	% change due to mush	42.18%*	91.67%	83.61%*	50.70%*	54.33%*
10%	Initial	44.24±0.11 [a]	0.044±0.0[a]	1.84±0.01 [a]	25.4±0.01 [a]	13.89±0.07 [a]
	W/out Mush	19.08±0.06 [b]	0.021±0.0 [b]	0.08±0.0 [b]	17.77±0.16 [b]	11.01±0.24 [b]
	With Mush	12.53±0.36 [c]	0.002±0.0 [c]	0.06±0.01 [c]	8.45±0.12 [c]	5.76±0.34 [c]
	% change due to mush	34.33%*	90.48%	25.00%	52.45%*	47.68%*
30%	Initial	47.12±0.35 [a]	0.067±0.0 [a]	1.41±0.02 [a]	41.94±0.50 [a]	14.72±0.08 [a]
	W/out Mush	22.89±0.13 [b]	0.027±0.0 [b]	0.16±0.01 [b]	29.18±0.07 [b]	12.41±0.12 [b]
	With Mush	16.52±0.25 [c]	0.003±0.0 [c]	0.09±0.01 [c]	11.37±0.01 [c]	6.83±0.16 [c]
	% change due to mush	27.83% [*]	88.89%	43.75%	61.03%*	44.96%*
60%	Initial	49.33±0.12 [a]	0.089±0.0 [a]	0.81±0.02 [a]	54.2±0.02 [a]	17.63±0.19 [a]
	W/out Mush	20.50±0.39 [b]	0.041±0.0 [b]	0.23±0.01 [b]	27.66±0.5 [b]	12.91±0.13 [b]
	With Mush	14.68±0.21 [c]	0.013±0.0 [c]	0.12±0.01 [c]	15.28±0.17 [c]	8.55±0.41 [c]
	% change due to mush	28.39%*	68.29%	47.83%	44.76%*	33.77%*

Values in the same column followed by different letter are significantly different according to Duncan Multiple Range test (P<0.05)

*Denote Change significant difference between incubated with and without mushroom (P<0.05)

Total Pesticide Soil Concentration

The pesticide concentration of the soil at day 0 was least in the 10% contaminated soil, while it is highest in the 60% contaminated soil (**Table 4**). However, the concentrations reduced after 90 days. In the same vein the decrease in concentration was more pronounced in soil that was incubated with *P. ostreatus.* Furthermore, the volume of pesticide lost to remediation was highest in the most contaminated soil (60%) sample and lowest was observed in the least contaminated (10%) soil. Additionally, the increased loss of pesticides in the soil was significantly associated with being incubated with mushroom for 90 days for all contaminant concentration.

Table 4: Total Pesticide Soil Concentration

Concentration	TOTAL PESTICIDE CONCENTRATION				
	Initial	W/out mush (FW)	Percentage Lost due to Natural attenuation	With Mush (FWM)	Percentage Lost due to mushroom
10%	11.263	8.912	20.87%	3.766	57.74%*
30%	24.086	15.165	37.04%	7.743	48.94%*
60%	42.574	32.744	23.09%	22.384	31.64%*

*Denote Change significant difference between incubated with and without mushroom (P<0.05)

Discussion and Conclusion

Bioaccumulation of metals, such as cadmium, caesium and zinc by several fungi has been reported (Gadd, 2001). Soil contaminated with mixture of hydrocarbons for 62-days (2-month), in the study showed a reduction in manganese, zinc, copper and lead (Gadd, 2001). This is similar to the finding of Adenipekun and Fasidi (2005) who reported that in soil contaminated with crude oil, fermented with *L. subnudus* and incubated for 3months, a reduction in iron and copper contents was observed at 10% crude oil contamination. The result of Adenipekun and Fasidi (2005) shows a total clean-up of lead by *Pleurotus pulmonarius* from the contaminated soil after 62-days of incubation while Adenipekun and Omoruyi (2008) reported that lead content decreased throughout the incubation period of 2 months from 108ppm to 84ppm, then finally to 40ppm, in the black-oil polluted soil after incubation with *Pleurotus ostreatus* after 1 and 2 months. The present work also shows the ability of the white-rot fungus, *Pleurotus ostreatus* to clean up manganese, zinc, copper and iron. Also the fungus was able to totally clean up the lead contamination in the soil, reducing the concentration to well below 0.02 mg/kg in all concentrations.

Furthermore, the results of the study represented the ability of *P. ostreatus* in degrading the pesticide substance contained in the sniper pesticide brand. After 90 days of incubation the fungus was able to reduce the concentration of pesticide in the soil by 57.74% in the 10% concentration and by 31.64% in the 60% concentration. This is in agreement with a study done by Hestbjerg *et al.* (2003). The study confirmed the ability of *P.ostreatus* to degrade a pesticide substance called lindane, the fungus reduced the concentration of the pesticide substance from 345 to 30 mg/l, within 45 day. Hussain *et al.* (2009) found that the fungus reduced the lindane concentrations in the soil from 558 to 37 mg/l in 274 days. Hussain *et al.* (2011) also reported a study where *P. ostreatus* reduced the concentration of lindane by 97% from initial concentration ranges of 7.1 to 37 mg/l, averaging 21 mg/l to 0.57 mg/l after 24 months of treatment.

The results of this study show that the contamination of soil samples with Sniper pesticide has a significant effect on the soil organic, chemical and nutrient parameters. All of which might hold important consequences in the agricultural and domestic use of the soil. Importantly, the study demonstrates on a small scale that the spawn of *Pleurotus ostreatus* can be useful in the bioremediation of soils contaminated with pesticides containing DDVP. It is thus logical to further explore the suitability of *Pleurotus ostreatus* in remediating this pesticide in natural conditions away from the controlled laboratory environment. The success of such field tests will have a clear positive impact on reducing inherent environmental soil pollution.

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