

Mycoremediation of Dichlorvos Pesticide Contaminated Soil by *Pleurotus pulmonarius* (Fries) Quelet

Njoku, K. L., Ulu, Z. *, Adesuyi, A. A., Jolaoso, A.O. and Akinola, M.O

Environmental Biology Research Unit, Cell Biology and Genetics Department,
University of Lagos, Akoka, Lagos, Nigeria

Received: 25.01.2018

Accepted: 22.04.2018

ABSTRACT: The extensive use of pesticides leads to accumulation of a huge amount of residues in the environment. As such, the present study investigates the potentiality of *Pleurotus pulmonarius* to bioremediate dichlorvos pesticides (2,2-dichlorovinyl dimethylphosphate) in contaminated soil. DDVP-polluted soils have been contaminated in five concentrations (5% v/w, 10% v/w, 15% v/w, 20% v/w, and 25% v/w), and the soil samples have been inoculated and incubated with pure culture of growing spawns of *P. pulmonarius*, obtained from commercial mushroom laboratory of Federal Institute of Industrial Research Oshodi, Lagos. The control, however, has not been inoculated. Each treatment has been in triplicates with the soils, analyzed for total amount of DDVP at day 0 and day 60, using gas chromatography and mass spectrometry. Also, pH, moisture content, and total organic matter of the soil have been determined. Results show that the rate of DDVP degradation in the soils with *Pleurotus pulmonarius* has been higher than the soil samples without mushroom after 60 days. However, for the control without mushroom (loss due to natural attenuation) and those inoculated with *P. pulmonarius* (bioremediation) the loss percentage of DDVP ascended with the percentage of pesticide from 5% to 25%. The DDVP loss across all different concentrations of mushroom inoculation have been significant ($p < 0.05$); however, for natural attenuation, it has not been significant ($p > 0.05$), except for the lowest pesticide level (5%). Activities of mycelia have decreased soil pH, moisture content, and total organic matter. There has been a very minimal pesticide bioaccumulation in mushroom tissue, which has not been significant ($p > 0.05$), but considerable at $p < 0.001$, indicating that *P. pulmonarius* has the potential to degrade DDVP pesticides in soil.

Keywords: Mycoremediation, *Pleurotus pulmonarius*, pesticides, Dichlorvos, mushroom

INTRODUCTION

Pesticides are organic compounds, manufactured and used for pest control (Agarry *et al.*, 2013). They can be utilized to prevent, destroy, repel, or mitigate any kind of pest (such as insects, mites, nematodes, weeds, rats, etc.), and include insecticides, herbicides, fungicides, and various other substances for pest control (U.S EPA, 2007). The use of pesticide in

Nigeria has increased ever since its introduction in early fifties for cocoa production. It has been estimated that about 125,000 - 130,000 metric tons of pesticides are being applied in Nigeria every year (Asogwa and Dongo, 2009). The extensive use of pesticides leads to an accumulation of huge amounts of residues in the environment, thus causing a substantial environmental health hazard due to the uptake and accumulation of these toxic

*Corresponding Author, Email: biologistalex@gmail.com

compounds in the food chain and drinking water (Njoku *et al.*, 2017). Adesuyi *et al* (2015) also linked phosphate and nitrate pollution in Nwaja creek to runoffs from adjoining agrarian lands as a result of pesticides and fertilizers application. Pesticide pollution can also result in reduced biodiversity and depression in soil heterotrophic bacteria (including the denitrifying ones), as well as the fungi (Ahmed *et al.*, 1998). Inadequate management practices specifically involving on-farm handling of pesticides appear to be a major source of pesticide contamination (Kuo and Regan, 1998), comprising the quality of soils, ground water, continental and coastal waters as well as the air (Surekha *et al.*, 2008).

Pesticides are toxic to many organisms, threatening the safety of ground and surface water. The contamination of surface and ground water with pesticides has many adverse impacts on the surrounding ecosystem. Organochlorine and organophosphate compounds stimulate the nervous system, causing tremors, irritability, and convulsions (Fragoero, 2005; ATSDR, 2005). Chronic exposure to organophosphates can not only destroy nerve fibers (neuropathy) but damage muscle tissues (myopathy) (Fragoero, 2005; US EPA, 2006).

Pesticides can be classified into six chemical groups, namely organochlorine, organophosphate, carbamate, synthetic pyrethroids, avermectin, and formamidine (Agarry *et al.*, 2013). Dichlorvos, also known as DDVP (2,2-dichlorovinyl dimethylphosphate), is an organophosphate pesticide, applied as mosquito insecticide in Nigeria for decades. It is being sold under many trade names such as Vapona, Atgard, Sniper, Ota pia-pia, and Nuvan (ATSDR, 1997; Chedi and Aliyu, 2010).

When the concentration of a pesticide, its metabolites, or by-products is significantly excessive, remediation becomes quite

necessary to avoid migration to a more sensitive area of the environment (Kearney, 1998). The conventional methods, employed for the remediation of organophosphate-contaminated sites, are mainly chemical treatments, with recycling, pyrolysis, incineration, and landfills being less efficient and costly, capable of leading to the formation of toxic intermediate (Dua *et al.*, 2002). Hence, today we need a remediation technology, which can be 'economically-viable', 'environmentally-sustainable', and 'socially-acceptable' for their degradation and transformation (Alexander, 1999; Surekha *et al.*, 2008). Bioremediation is a more environmentally-friendly technique for remediation, compared to chemical treatment. It involves the transformation of simple or complex chemical compounds into non-hazardous forms via biological agents, resulting in materials of higher nutritive value or simply reducing the final bulk of the product (Furukawa, 2003). Mycoremediation is one of bioremediation methods, involving the use of fungi to clean up polluted sites (Nawaz *et al.*, 2011).

The white rot fungi *Pleurotus pulmonarius* is a wood-decaying basidiomycetes, capable of degrading not only lignin but also variable recalcitrant environmental pollutants due to its ability to secrete lignolytic enzymes like lignin peroxidase, manganese peroxidase, and laccases that aid the degradation process (Ogbo *et al.*, 2006). *Phanerochaete chrysosporium* is the first fungus to be associated with degradation of organopollutants, since it has been extensively studied as a model microorganism in researches on the mechanism of lignin degradation (Sasek, 2003). Reddy and Mathew (2001) showed that this species was able to degrade DDT, lindane, and atrazine. Additionally, *P.ostreatus* has been tested for lindane degradation and has been found to effectively reduce the concentrations from 345 to 30 mg/l within 45 days, in a bench-

scale test (Fragoieiro, 2005). Novotny *et al.* (1999) also described *P. ostreatus* as a suitable candidate to apply for cleaning up the soil samples, contaminated with recalcitrant pollutants, thanks to its capability of robust growth and efficient extracellular enzyme production in the soil, even in the presence of such pollutants as PAHs. They suggested that mycelial growth through contaminated soil and efficient enzyme expression were the key factors for removal of pollutant molecules from the bulk soil. Adenipekun *et al.* (2013) found *P. pulmonarius* as a bioremediator of soil, contaminated with diesel oil, observing that the remediated soil was able to support the growth of *Corchorus olitorius* at all levels of contamination. Okparanma *et al.* (2011) stated that spent white-rot fungi (*Pleurotus ostreatus*) substrate can be used to biotreat Nigerian oil-based drill cuttings, containing polyaromatic hydrocarbons (PAH's), in laboratory conditions. Njoku *et al.* (2016) observed that white-rot fungus (*Pleurotus pulmonarius*) was potential not only to degrade petroleum hydrocarbons but to transform them into simpler and less hazardous compounds also. Adelaja *et al.* (2017) recommended that soil, contaminated with the DDVP pesticides, may be treated with *P. ostreatus* to reduce heavy metal contents and total pesticide concentration. The objective of this research work is to investigate the potentiality and effectiveness of *Pleurotus pulmonarius* in remediation of soil, contaminated with organophosphate pesticides (Dichlorvos) and to observe the effects of the degradation process on soil pH, moisture content, and nutrient status.

MATERIAL AND METHODS

The top soil, used in this experiment, was collected within a depth of 1 to 10 cm from the nursery site of the Botanical garden of the University of Lagos, Akoka, Lagos State, Nigeria. The soil was sieved with a 2-mm mesh to remove debris. Pure culture of *P. pulmonarius* was obtained from a

commercial mushroom laboratory at Federal Institute of Industrial Research Oshodi (F.I.I.R.O.) Lagos, Nigeria with the analytical grade of dichlorvos (2,2-dichlorovinyl dimethylphosphate, 97% pure) (made by Sigma-Aldrich, USA), used for the research, being purchased from a chemical store in Lagos State.

In order to achieve DDVP pollutions of 5%, 10%, 15%, 20%, and 25% v/w, 100g of the sample soil was measured into each pot and treated with 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml of DDVP pesticide, respectively. Each treatment was replicated three times, with the control samples not receiving any. The remediation experiment was conducted inside the mushroom Laboratory at Federal Institute of Industrial Research Oshodi Lagos, adopting modified methods of Quimio *et al.*, (1990) and Adenipekun and Kassim (2006). The prepared soil concentrations were mixed thoroughly with 40 g of moistened clean rice bran, while 40 g of saw dust was mixed with the contaminated soil in each bag. Calcium carbonate (CaCO₃) and calcium sulphate (CaSO₄) were added as pH buffer as well as calcium and carbon sources to the substrate, to be mixed with the experimentally-contaminated soil. The bagged pesticide-contaminated substrates were pasteurized at 100⁰C for 3hrs and left in the pasteurization drum to cool to be removed the following day (Quimio *et al.*, 1990). As much as 20 g of vigorously growing spawn of *Pleurotus pulmonarius* was inoculated to the bagged substrates and incubated for 60 days until mycelial colonization was complete (Oei, 1996). The substrate bags were initiated for fruition through exposure to light, 90% humidity, and water that was sprayed daily (Quimio *et al.*, 1990). The polyethylene bags were completely removed and the substrates were exposed and watered, with the watering stopped after the appearance of basidiocarp.

The protocol, followed for the extraction of DDVP in substrate, was outlined by La Dreau *et al.*, (1997). Firstly, 10.00g of the sample was weighed in an amber bottle and 20ml of dichloromethane was added to it. Both got shaken vigorously for 30 minutes, using a mechanical shaker. Afterwards, it was filtered into a glass beaker and 1ml of the filtrate got transferred into a simple vial to be stored in a refrigerator for future analysis. One ml of pure form of DDVP was first injected into the Gas chromatography–mass spectrometry (GC-MS) to obtain a standard chromatogram

and peak area. The reason for this was to calibrate the GC-MS for the test sample. Then, 1ml of the test sample was injected into the GC-MS as well, to obtain equivalent chromatogram and peak area. Finally, the peak area of the test sample got compared to that of the standard, with respect to the concentration of the standard to get the concentration of the test sample (Hernandez *et al.*, 2005).

Sum peak areas of two known values were used to obtain the unknown concentration of test sample, putting the standard concentration into equation (1) (Smalling and Kuivila, 2008).

$$\text{Conc.of test sample} = \frac{\text{Sum of peak area of sample} \times \text{concentration of standard}}{\text{Sum of peak area of standard}} \quad (1)$$

(where standard concentration was 99.91 mg/kg and Standard peak area, 8431.7818)

The percentage of DDVP lost/degraded in the soil was determined, using the formula below:

$$\text{Percentage loss (remediation)} = \frac{\text{Initial DDVP} - \text{Final DDVP}}{\text{Initial DDVP}} \times 100\%$$

The mushrooms from each bag with each concentration were collected and were dried on blotting paper, cut into pieces, and oven-dried at 105°C for 24hrs (Udochukwu *et al.*, 2014). Dried samples were homogenized, using a blender, into fine powder and stored in pre-cleaned polyethylene bottles, prior to analysis by Gas Chromatography (GC-MS), as was done for the determination of DDVP level in the substrate.

Soil pH was measured on day 0 (initial) as well as day 60 (final). The pH of the soil samples was determined according to the procedure, developed by Bates (1954), wherein Twenty grams of soil sample was weighed into a 50 mL beaker and 20 mL of deionized distilled water got added to it. The mixture got stirred manually for 5 minutes and allowed to settle down for 30 minutes, so that the pH could be measured by means of a pH meter 3015 (Jenway,

U.K.). The moisture content and total organic matter of the soil were determined, using the method of Association of Official Analytical Chemists (A.O.A.C, 2003).

Results from the laboratory analyses were subject to descriptive statistics (mean and standard error of mean) and analysis of variance (two-way ANOVA) at 95%, 99%, and 99.9% confidence level along with Duncan's multiple range test, using GraphPad prism 7.0.

RESULTS AND DISCUSSION

It was observed that *P. pulmonarius* grew and survived in all various amounts of DDVP pesticides, added to the soil samples. The ability of white rot fungi to grow on pesticide at different levels of contamination through their mycelia colonization conformed with the results obtained by Aust and Swanner (2003), who reported that white rot fungi could withstand toxic concentrations of most organo-pollutants and grow well in them. Njoku *et al.* (2016) also demonstrated that *P. pulmonarius* were able to grow optimally in the presence of harmful contaminants such as petroleum products (petrol, diesel, and new or spent engine oil), able to detoxify such contaminants.

The total DDVP level in the soils were higher on day 0 (initial level) than the final one (day 62). The initial level of DDVP pesticides in the soil samples rose as the level of pesticides added to the soil was increased (Table 1). This trend was also observed in the final level of DDVP in the soils with *P. pulmonarius* and control. In the control sets, i.e., DDVP-contaminated soil without *P. pulmonarius* (loss due to natural attenuation), the DDVP residue dropped from the initial level of 81.61 ± 0.17 mg/kg to 69.33 ± 0.03 mg/kg, for 5% DDVP contamination; from 95.09 ± 0.05 mg/kg to 81.58 ± 0.10 mg/kg for 10% contamination; from 124.78 ± 0.13 mg/kg to 100.77 ± 0.13 mg/kg for 15% contamination; from 142.34 ± 0.07 mg/kg to 116.10 ± 0.05 mg/kg for 20% contamination; and from 161.79 ± 0.12 mg/kg to 123.10 ± 0.08 mg/kg for 25% contamination. As for the recorded losses of percentage, they were 15.05% for 5% contamination, 14.21% for 10% contamination, 19.24% for 15% contamination, 18.44% for 20% contamination, and 23.91% for 25% contamination. The DDVP loss in the control sets were not significant ($p > 0.05$) except for the least pesticide level (5%). However, for soils samples inoculated with *P. pulmonarius* (bioremediation) the percentage losses of DDVP recorded were 81.25% for 5% contamination (from initial 81.61 ± 0.17 mg/kg to 15.30 ± 0.17 mg/kg), 87.62% for 10% contamination (from initial 95.09 ± 0.05 mg/kg to 11.77 ± 0.12 mg/kg), 92.52% for 15% contamination (124.78 ± 0.13 mg/kg reduces to 9.33 ± 0.04 mg/kg), 95.00% for 20% contamination (142.34 ± 0.07 mg/kg reduces to 7.11 ± 0.03), and 96.43% for 25% DDVP contamination (161.79 ± 0.12 mg/kg reduces to 5.78 ± 0.05 mg/kg). The highest loss of DDVP was observed to be in the highest volume of contamination (25%). The DDVP loss due to *P. pulmonarius* inoculation was significant ($p < 0.05$) for all the different volumes of added DDVP.

White-rot fungi are becoming recognized for their ability to efficiently

biodegrade numerous toxic contaminants (Osano *et al.*, 1999; Bending *et al.*, 2002; Adenipekun *et al.*, 2011). Previous laboratory studies focused on the ability of *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Pleurotus ostreatus*, *Phellinus weirii*, and *Polyporus versicolor* to degrade persistent compounds and pesticides (Safferman *et al.*, 1995; Osano *et al.*, 1999). Bending *et al.* (2002) reported that nine different species of white rot fungi were able to degrade different types of pesticides. They saw that these fungi were effective, as they produced extracellular enzymes, catalysing a reaction that could degrade lignin, an aromatic plant compound (Fragoero, 2005). It was also observed that similar mechanisms were involved in the degradation of all different pesticides (Bending *et al.*, 2002). This ability to degrade a wide variety of compounds was attributed, at least in part, to the action of lignolytic enzymes (Fragoero, 2005; Rigas *et al.*, 2007). The present study showed that *P. pulmonarius* also possessed such a remediating ability to degrade dichlorvos pesticides. Veignie *et al.* (2004) found out that *P. pulmonarius* had extracellular lignin modifying enzymes (LMEs) with very low substrate specificity, confirming their ability to mineralize a wide range of organopollutants.

P. pulmonarius (filamentous fungi) possess some attributes, enabling them to act as good potential agents of degradation via ramifying the substratum and digesting it through the secretion of extracellular enzymes, which are nonspecific (Adenipekun *et al.*, 2011). The branching filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil. Fungal degradation involves breakdown of organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals (Haritash and Kaushik, 2009).

The bioaccumulations of DDVP in the tissues of *P. pulmonarius* were all very minimal and low ranging from 3.39±0.03 mg/kg (5% contamination) to 6.68±0.05 mg/kg (25% contamination). Bioaccumulations of DDVP were not significant at p>0.05, but quite considerable at p<0.001. There was no fruition of *P. pulmonarius* in 10% and 20% DDVP contaminated soils. According to Kulshreshtha et al. (2014) toxicity level in the fruiting bodies is based on two facts: biodegradation and

biosorption. Mushroom possesses the suitable enzymatic machinery for biodegradation, which leads to the degradation of pollutants from the substrate and its conversion into less toxic products, rendering the fruiting bodies safe for consumption (Gruter et al., 1991; Kulshreshtha et al, 2013). Absorption of DDVP pesticides by *P. pulmonarius* makes them unsuitable for consumption; however, more inoculation time could reduce the accumulated pollutants.

Table 1. Amount of DDVP (mg/kg) in the contaminated soil, inoculated with *Pleurotus pulmonarius* (values in bracket show the percentage loss from the initial values)

Pesticide Level		Level of DDVP (mg/kg)
5%	Initial DDVP level	81.61 ± 0.17
	Final DDVP level in soil without <i>P. pulmonarius</i>	69.33 ± 0.03* (15.05%)
	Final DDVP level in soil with <i>P. pulmonarius</i>	15.30 ± 0.17** (81.25%)
	Accumulation of DDVP in <i>P. pulmonarius</i>	3.39 ± 0.03***
10%	Initial DDVP level	95.09 ± 0.05
	Final DDVP level in soil without <i>P. pulmonarius</i>	81.58 ± 0.10 (14.21%)
	Final DDVP level in soil with <i>P. pulmonarius</i>	11.77 ± 0.12** (87.62%)
	Accumulation of DDVP in <i>P. pulmonarius</i>	N.A
15%	Initial DDVP level	124.78 ± 0.13
	Final DDVP level in soil without <i>P. pulmonarius</i>	100.77 ± 0.13 (19.24%)
	Final DDVP level in soil with <i>P. pulmonarius</i>	9.33 ± 0.04** (92.52%)
	Accumulation of DDVP in <i>P. pulmonarius</i>	4.02 ± 0.02***
20%	Initial DDVP level	142.34 ± 0.07
	Final DDVP level in soil without <i>P. pulmonarius</i>	116.1 ± 0.05 (18.44%)**
	Final DDVP level in soil with <i>P. pulmonarius</i>	7.11 ± 0.03 (95.00%)**
	Accumulation of DDVP in <i>P. pulmonarius</i>	N.A
25%	Initial DDVP level	161.79 ± 0.12
	Final DDVP level in soil without <i>P. pulmonarius</i>	123.1 ± 0.08 (23.91%)
	Final DDVP level in soil with <i>P. pulmonarius</i>	5.78 ± 0.05*** (96.43%)
	Accumulation of DDVP in <i>P. pulmonarius</i>	6.68 ± 0.05***

*Significant at p < 0.05; **significant at p < 0.01, ***significant at p < 0.001. N.A – Not Analysed (due to lack of fruiting).

Table 2 presents the pH, moisture content, and total organic matter content of the soil, incubated with *P. pulmonarius* and the control, showing that the pH values rose as the volume of DDVP pesticides in the contaminated soil increased. As observed in this study, there was a reduction in the pH of soil samples, contaminated with DDVP pesticides, having no *P. Pulmonarius*, along with a further significant reduction in the DDVP-contaminated soil, inoculated with *P. pulmonarius*. The highest impact of *P.*

pulmonarius on soil pH was noticed in samples with 25% DDVP treatment (8.03%), followed by the ones with 20% treatment (5.51%). Coincidentally, these were contamination levels, in which pesticides loss (degradation) was prominent, which could be seen as suggestive of contamination levels where the fungus had more metabolic and physiological activities to produce substances, modifying the substrate's pH (Adenipekun and Isikhuemhen, 2008). White rot fungi are known to

secrete organic acid into their substrate, presumably capable of lowering the pH to optimum levels for their enzymes to function best (Hofrichter *et al.*, 1999; Hossain and Anatharaman, 2006). Hence, it plays a vital role in biodegradation activities of fungi (Njoku *et al.*, 2016). The effectiveness and degradation of herbicides and insecticides has also been reported to be pH dependent (USDA, 2011). Njoku *et al.* (2016) observed that the pH of soil, contaminated with petroleum products, incubated with *P. pulmonarius* decreased from 6.84 to 6.29 in the highest concentration of contaminant (11.28%). Similar findings by Adenipekun and Fasidi (2005) also showed a decrease of pH value from 6.90 to 6.62 and finally to 6.25 after 3 and 6 months of incubation, respectively, with *Lentinus subnudus*, a Nigerian white rot fungus. Adenipekun and Omoruyi (2008) also observed that the pH of cement-contaminated soil, incubated with *P. ostreatus*, decreased from 7.55 in the control to 7.54 and 7.11 after one and two months. For diesel-contaminated and battery-contaminated soils, the pH declined from 5.90 in the control to 4.68 after one month of incubation.

Similarly, the initial moisture and organic matter contents of the soil ascended with the increase in the concentration of DDVP pesticides, added to the soil; however, the moisture and total organic matter content of the soil descended due to activities of *P. pulmonarius*. The highest impact of *P. pulmonarius* on the moisture content was also observed to be in soil samples with 25% treatment (16.00%), while the least one belonged to those with 5% treatment concentration (7.34%). Matric potential is known to affect physiological activity of soil microorganisms (Zak *et al.*, 1999) and different fungi may have optimal biodegradation rates at different water availabilities, as reported by Fragoiro (2005). Availability of water in soil may be

a very important factor, affecting the success of bioremediation, since it affects oxygen supply and, consequently, fungal growth and enzyme production (Marin *et al.*, 1998; Philippousis *et al.*, 2001). Moreover, apart from affecting fungi physiology, water availability also affects pesticide binding and distribution in the soil. The behaviour of organic compounds in water plays a very significant role on their availability for bioutilization in the environment (Atagana *et al.*, 2003). Pesticide degradation is known to be slow in dry soils, hence the rate of pesticide transformation and degradation generally increases with water content (Pal *et al.*, 2006). Pesticides with low water solubility tend to be more resistant to biodegradation than compounds of higher water solubility. Fungi and other microorganisms can use only the dissolved fraction of the compound in soil solution (Cork and Krueger, 1991).

The highest impact of *P. pulmonarius* on the organic matter content of the soil was observed in soil with 25% treatment (63.83%), while the lowest belonged to soil with 5% treatment concentration (52.71%). At the end of the study, the organic matter content declined as the incubation period was increased from 0 to 60 days. The oxidation of organic matter by aerobic organisms resulted in the production of carbon dioxide (Harris and Steer, 2003). Respiration may increase in response to an increase in organisms biomass or as a result of the increased activity of a stable biomass (Harris and Steer, 2003; Fragoiro, 2005). The result of this study was in agreement with Wackett and Hershberger (2001), who concluded that biodegradation rate depended on soil conditions (which included temperature, aeration, pH, and organic matter content) as well as the frequency of pesticide application (volume).

Table 2. Impact of fungal activity on physicochemical parameters of soil samples, contaminated with DDVP pesticides

Amount of DDVP Pesticides added		pH	Moisture Content (%)	Total Organic Matter (%)
5%	Initial DDVP level	6.63 ± 0.02	6.95 ± 0.01	67.85 ± 0.04
	Final DDVP level in soil w/out <i>P. pulmonarius</i>	6.56 ± 0.03	6.95 ± 0.03	56.91 ± 0.11
	Final DDVP level in soil with <i>P. pulmonarius</i>	6.44 ± 0.03	6.44 ± 0.03*	32.09 ± 0.24*
	% change Due to Mushroom	2.87	7.34	52.71
10%	Initial DDVP level	6.64 ± 0.023	7.07 ± 0.017	67.35 ± 0.03
	Final DDVP level in soil w/out <i>P. pulmonarius</i>	6.57 ± 0.04	6.91 ± 0.03	56.02 ± 0.28
	Final DDVP level in soil with <i>P. pulmonarius</i>	6.42 ± 0.03	6.42 ± 0.03*	30.81 ± 0.11*
	% change Due to Mushroom	3.31	9.19	54.25
15%	Initial DDVP level	6.70 ± 0.00	7.10 ± 0.012	67.31 ± 0.06
	Final DDVP level in soil w/out <i>P. pulmonarius</i>	6.60 ± 0.06	6.87 ± 0.05	54.84 ± 0.03
	Final DDVP level in soil with <i>P. pulmonarius</i>	6.39 ± 0.03	6.39 ± 0.03*	28.99 ± 0.18*
	% change Due to Mushroom	4.63	10.00	56.93
20%	Initial DDVP level	6.72 ± 0.012	7.35 ± 0.012	66.85 ± 0.017
	Final DDVP level in soil w/out <i>P. pulmonarius</i>	6.63 ± 0.04	6.86 ± 0.03	52.19 ± 0.11
	Final DDVP level in soil with <i>P. pulmonarius</i>	6.35 ± 0.03*	6.35 ± 0.03*	28.40 ± 0.17*
	% change Due to Mushroom	5.51	13.61	57.52
25%	Initial DDVP level	6.85 ± 0.012	7.50 ± 0.012	66.72 ± 0.012
	Final DDVP level in soil w/out <i>P. pulmonarius</i>	6.62 ± 0.02	6.80 ± 0.03	51.08 ± 0.05
	Final DDVP level in soil with <i>P. pulmonarius</i>	6.30 ± 0.03*	6.30 ± 0.03*	24.13 ± 0.10*
	% change Due to Mushroom	8.03	16.00	63.83

* Significant at $p < 0.05$

CONCLUSION

The present study showed that *P. pulmonarius* was capable of growing and utilizing Dichlorvos in soil, being quite effective for remediation in a controlled laboratory environment. Thus *P. pulmonarius* can be further explored for bioremediation of pesticide-contaminated soils. Bioaccumulation of pesticides in mushroom tissue were low and minimal; however, more inoculation time could enhance further utilization of pollutants, thus reducing the accumulated residue.

REFERENCES

- Adelaja, O.D., Njoku, K.L. and Akinola, M.O. (2017) Mycoremediation of Pesticide Contaminated Soil Using Mushroom *Pleurotus ostreatus*. *International Journal of Health and Economic Development*, 3(2), 20-27.
- Adenipekun, C.O., Ayanleye, O.O. and Oyetunji, O.J. (2013). Bioremediation of soil contaminated by diesel oil using *Pleurotus pulmonarius* Fries (Quelet) and its effects on the growth of *Corchorus olitorius* (L.). *Journal of Applied Biosciences*, 68: 5366 – 5373.
- Adenipekun, C.O. and Fasidi, I.O. (2005).

Degradation of selected agricultural wastes by *Pleurotus tuber-regium* (Fries) Singer and *Lentinus subnudus* (Berk) – Nigerian Edible Mushrooms. *Advances in Food Science*, 27(2): 61–64.

Adenipekun, C.O. and Isikhuemhen, O.S. (2008). Bioremediation of Engine Oil Polluted Soil by the Tropical White Rot Fungus, *Lentinus squarrosulus* Mont. (Singer). *Pakistan Journal of Biological Sciences*, 11: 1634-1637.

Adenipekun, C.O. and Kassim, L.Q. (2006). Effects of spent engine oil on some growth parameters and moisture content of *Celosia argentea* L. *Nigerian Journal of Botany*, 19(2):318-324.

Adenipekun C.O., Olanrewaju, O.O. and Ogunjobi, A. A. (2011). Bioaccumulation of Heavy metals and Nutrient content Supplementation by two White rot fungi in Crude oil polluted soils. *Researcher*, 3(5):13-20.

Adenipekun, C.O. and Omoruyi, O.M. (2008). Bioremediation of contaminated soils by *Pleurotus ostreatus* (Jacq. Fr.) P. Kumm. *Nigerian Journal of Botany*, 21(2): 274–279..

Adesuyi, A.A., Nnodu, V.C., Njoku, K.L. and Jolaoso, A. (2015). Nitrate and Phosphate Pollution in Surface Water of Nwaja Creek, Port Harcourt, Niger Delta, Nigeria. *International Journal of Geology, Agriculture and Environmental Sciences*, 3(5), 14-20.

- Agarry, S.E., Olu-Arotiowa, O.A., Aremu, M.O. and Jimoda, L.A. (2013). Biodegradation of Dichlorovos (Organophosphate Pesticide) in Soil by Bacterial Isolates. *Journal of Natural Sciences Research*, 3(8): 12-16.
- Ahmed, M., Ismail, S. and Mabrouk, S. (1998). Residues of some chlorinated hydrocarbon pesticides in rain water, soil and ground water, and their influence on some soil microorganisms. *Environmental International*, 24(5-6): 665-670.
- Alexander, M. (1999). Biodegradation and Bioremediation. 2nd Edition. Academic Press: New York, NY.
- Asogwa, E.U. and Dongo, L.N. (2009). Problems associated with pesticide usage and application in Nigerian cocoa production: A review. *African Journal Agricultural Research*, 4(8): 675-683.
- Atagana, H., Haynes, R. and Wallis, F. (2003). The use of surfactants as possible enhancers in bioremediation of creosote contaminated soil. *Water, Air and Soil Pollution*; 142: 137-149.
- ATSDR (1997). Toxicological profile for dichlorvos. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry (ATSDR), pp. 99-110.
- ATSDR (2005). Toxicological profile for Hexachlorocyclohexane. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Agency for Toxic Substances and Disease Registry (ATSDR),
- Aust, S.D. and Swanner, P.R. (2003). Detoxification and Metabolism of chemicals by white-rot fungi .In: Pesticide decontamination and detoxification. J.J.P.C., Zhu. S.D. Aust. T Lemley Gan, 3-14. Washington, D.C. Oxford University Press.
- Bates, R.A. (1954). *Electrometric determination*. John Wiley Sons, Inc. New York.
- Bending, G.D., Friloux, M. and Walker, A. (2002). Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. *FEMS Microbiology Letters*, 18:212(1):59 – 63.
- Chedi, B. A. Z. and Aliyu, M. (2010). Effect and Management of Acute Dichlorvos Poisoning in Wistar Rats. *Bayero Journal of Pure and Applied Sciences*, 3(2): 1 – 3.
- Cork, D.J. and Krueger, J.P. (1991). Microbial transformation of herbicides and pesticides. *Advances in Applied Microbiology*, 36: 1-66.
- Dua, M., Singh, A., Sethunathan, N. and Johri, A.K. (2002). Biotechnology and bioremediation: successes and limitations. *Applied Microbiology and Biotechnology*, 59(2-3): 143-52.
- Fragoieiro, S. I. S. (2005). Use of Fungi in Bioremediation of Pesticides. Ph.D Dissertation, Cranfield University.
- Furukawa, K. (2003). Super bugs for Bioremediation. *Trends in Biotechnology*, 21: 187- 190.
- Gruter, A., Friederich, U. and Wurgler, F.E. (1991). The mutagenicity of edible mushrooms in a histidine-independent bacterial test system. *Food Chemistry*, 4:159–165.
- Haritash, A.K. and Kaushik, C.P. (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *Journal of Hazard Mater*, 169:1–15.
- Harris, J. and Steer, J. (2003). Modern methods for estimating soil microbial mass and diversity: and integrated approach. In Sasek V. *et al.* (eds.) The utilization of bioremediation to reduce soil contamination: Problems and solutions, pp. 29-48. Kluwer Academic Publishers.
- Hernanadez, F. Sancho, J. and Pozo, O. (2005). Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. *Analytical and Bioanalytical Chemistry* 382(4):934-946.
- Hofrichter, M., Vare, T., Kalsi, M., Galkin, S., Scheibner, K., Fritsche, W. and Hatakka, A. (1999). Production manganese peroxidase and organic acids and mineralization of ¹⁴C-labelled lignin (14C-DHP) during solid-state fermentation of wheat straw with the white ofrot fungus *Nematoloma frowardii*. *Applied Environmental Microbiology*, 65: 1864-1870.
- Hossain, M.S. and Anantharaman, N. (2006). Activity Enhancement of Ligninolytic Enzyme of *Trametes versicolor* with Bagasse Powder. *African Journal of Biotechnology*, 5:189-194.
- Kearney, P. and Wauchope, R. (1998). Disposal options based on properties of pesticides in soil and water. In: Kearney P. and Roberts T. (Eds.) Pesticide remediation in soils and water. Wiley Series in Agrochemicals and Plant Protection.
- Kulshreshtha, S., Mathur, N. and Bhatnagar, P. (2014). Mushroom as a product and their role in Mycoremediation. *AMB Express*, 4:29. doi: 10.1186/s13568-014-0029-8
- Kulshreshtha, S., Mathur, N. and Bhatnagar, P. (2013). Cultivation of *Pleurotus citrinopileatus* on handmade paper and cardboard industrial wastes. *Industrial Crop Production*, 2013;4:340–346.

- Kuo, W. and Regan, R. (1998) Aerobic carbamate bioremediation aided by compost residuals from the mushroom industry: laboratory studies. *Compost Science & Utilization*; 6 (1): 19-29.
- La Dreau, Y., Jacquot, F., Doumena, P., Guilliano, M., Bertrand, J. and Mille, G.(1997). Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum waste of a refinery from 1956-1992. *Marine Pollution Bulletin* 34: 456-468.
- Marin, S., Sanchis, V., Ramos, A. and Morgan, N. (1998). Effect of water activity on hydrolytic enzyme production by *Fusarium moniliforme* and *Fusarium proliferatum* during colonization of maize. *International Journal of Food Microbiology*, 42(3):185 – 194.
- Nawaz, K., Hussain, K., Choudary, N., Majeed, A., Ilyas, U., Ghani, A., Lin, F., Ali, K., Afgan, S., Ghulam, R. and Lasari, M.I. (2011). Eco-friendly role of biodegradation against agricultural pesticides hazards. *African Journal of Microbiology Research*, 5(3): 177-183.
- Nerud, F., Baldrian, J., Gabriel, J. and Ogbeifun, D. (2003). Nonenzymic degradation and decolorization of recalcitrant compounds. In Sasek V. et al. (Eds.) *The utilization of bioremediation to reduce soil contamination: Problems and solutions*, pp. 29-48. Kluwer Academic Publishers.
- Njoku, K.L., Yussuf, A., Akinola, M.O., Adesuyi, A.A., Jolaoso, A.O. and Adedokun, A.H. (2016). Mycoremediation of Petroleum hydrocarbon polluted soil by *Pleurotus pulmonarius*. *Ethiopia Journal Environmental Sciences and Management*, 9(Suppl. 1): 865 – 875.
- Njoku, K.L., Ezeh, C.V., Obidi, F.O. and Akinola, M.O. (2017). Assessment of Pesticide Residue Levels in Vegetables sold in some Markets in Lagos State, Nigeria. *Nigerian Journal of Biotechnology*, 32: 53-60.
- Novotny, C., Erbanova, P., Sasek, V., Kubatova, A., Cajthman, T., Lang, E., Krahl, J. and Zadrazil, F. (1999). Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi. *Biodegradation*; 10: 159-168.
- Oei, P. (1996). *Mushroom cultivation with special emphasis on appropriate techniques for developing countries*. Tool Publications, Leiden, The Netherlands, pp 94-119.
- Okeke, B., Smith, J., Paterson, A. and Watson-Craik, I. (1996). Influence of environmental parameters on pentachlorophenol biotransformation in soil by *Lentinula edodes* and *Phanerochaete chrysosporium*. *Applied Microbiology and Biotechnology*, 45:263-266.
- Okparanma, R.N., Ayotamuno, J.M., Davis, D.D. and Allagoa, M. (2011). Mycoremediation of polycyclic aromatic hydrocarbons (PAH) - contaminated oil-based drill-cuttings. *African Journal of Biotechnology*, 10(26):5149-5156.
- Osano, A.A., Siboe, G.M., Ochanda, J.O. and Kokaro, J.O. (1999). Biodegradation of DDT: The Role of *Pleurotus* sp., A Lingnicolous Fungus. *Bioremediation of Nitroaromatic and Haloaromatic Compounds*. B.C. Alleman and A. Leeson, Eds. Columbus, OH: Battelle Press.
- Philippoussis, A., Diamantopoulou, P., Euthimiadou, H. and Zervakis, G. (2011). The composition and porosity of lignocellulosic substrates influence mycelium growth and respiration rates of *Lentinus edodes*. *International Journal of Medicinal Mushroom*, 3(2-3): 198.
- Quimio, T.H., Chang, S.T. and Royse, D.J. (1990). Technical guidelines for mushrooms growing in the Tropic. *FAO Plant Production and Protection*, No. 106, Rome. pp152.
- Reddy, C. and Mathew, Z. (2001). Bioremediation potential of white rot fungi. In Gadd G. (Eds.) *Fungi in bioremediation*. Cambridge University Press. Cambridge, U.K.
- Rigas, F., Papadopoulou, K., Dritsa, V. and Doulia, D. (2007). Bioremediation of a soil contaminated by lindane utilizing the fungus *Ganoderma australe* via response surface methodology. *Journal of Hazardous Materials*, 140: 325–332.
- Safferman, S.I., Lamar, R.T., Vonderhaar, S., Neogy, R., Haught, R.C. and Krishnan, E.R. (1995). Treatability study using *Phanerochaete sordida* for the Bioremediation of DDT Contaminated Soil. *Toxicological and Environmental Chemistry*, 50: 237-251
- Sasek, V. (2003). Why mycoremediations have not yet come to practice. In : Sasek V. et al. (Eds.) *In: The utilization of bioremediation to reduce soil contamination: Problems and solutions*, pp. 247-276. Kluwer Academic Publishers, 2003.
- Smalling, K.L. and Kuivila, K.M. (2008). Multi-residue method for the analysis of 85 current use and legacy pesticides in bed and suspended sediments. *Journal of Chromatography A*, (1)8-18.
- Surekha, R.M., Lakshmi, P.K.L., Suvarnalatha, D., Jaya, M., Aruna, S., Jyothi, K., Narasimha, G. and Venkateswarlu, K. (2008). Isolation and characterization of a chlorpyrifos degrading bacterium from agricultural soil and its growth response. *African Journal of Microbiological Research*, 2: 26-31.

Udochukwu, U., Nekpen, B.O., Udinyiwe, O.C. and Omeje, F.I. (2014). Bioaccumulation of Heavy metals and pollutants by edible mushroom collected from Iselu market Benin-city. *Internationaal Journal of Current Microbiology and Applied Sciences*, 3(10): 52-57

USDA (2011). Soil Quality Kit – Guides for Educators. Retrieved online from: https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_053293.pdf. Accessed on 30th December, 2016.

Veignie, E., Rafin, C., Woisel, P. and Cazier, F. (2004). Preliminary evidence of the role of

hydrogen peroxide in the degradation of benzo[a]pyrene by a non-white rot fungus *Fusarium solani*. *Environmental Pollution*, 129: 1-4.

Wackett, L.P. and Hershberger, C.D. (2001). *Biocatalysis and Biodegradation: Microbial Transformation of Organic Compounds*. ASM Press, Washington DC. 228pp.

Zak, D., Holmes, W., MacDonald, N. and Pregitzer, K. (1999). Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Science Society of America Journal*, 63: 575-584.

