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Bioaccumulation potential of Pleurotus florida on Engine oil polluted soil using different substrates

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ABSTRACT

The accumulation of environmental pollutants in edible mushrooms has raised concerns regarding food safety and environmental sustainability. This study investigated the bioaccumulation of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in *Pleurotus florida* cultivated on different agricultural substrates grown on engine oil-polluted soil. The experiment was conducted in the laboratory of the Department of Public Health and Preventive Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Four substrates sawdust, sugarcane bagasse, rice straw, and *Andropogon* straw—were used for mushroom cultivation. The experimental design Completely Randomized Design (CRD) with four treatments replicated five times. Polluted soil collected from a mechanic workshop was incorporated at different levels (50 g, 100 g, 150 g, and 200 g) into 200 g of each pasteurized substrate and inoculated with spawn of *Pleurotus florida*. Growth parameters such as fresh weight, dry weight, no of fruiting bodies, stipe length, and cap diameter were recorded. Samples of the polluted soil and harvested mushrooms were analyzed to determine the concentrations of heavy metals and PAHs. The results revealed the presence of five heavy metals zinc (Zn), chromium (Cr), arsenic (As), cadmium (Cd), and copper (Cu) in the engine oil-polluted soil before cultivation. Significant ($P \leq 0.05$) accumulation of Zn, Cr, As, and Cd was detected in the mushroom fruiting bodies harvested from *Andropogon* straw, sugarcane bagasse, rice straw, and the control treatment. However, there was no significant difference in copper accumulation across the substrates and control. Analysis of PAHs showed detectable accumulation in mushrooms grown on most substrates, with the highest concentration of phenanthrene recorded in mushrooms cultivated on sugarcane bagasse (1.91 ± 0.00) and the lowest concentration of fluorene observed in mushrooms grown on sawdust (0.17 ± 0.00). No PAH accumulation was detected in the control treatment. The findings demonstrate that *Pleurotus florida* can accumulate heavy metals and PAHs from contaminated substrates, highlighting its potential for environmental remediation. However, the study also emphasizes the importance of substrate selection in mushroom cultivation to reduce the risk of contaminant accumulation in edible fruiting bodies. Among the substrates tested, sawdust proved to be the most suitable and safer substrate for commercial mushroom production.
Keywords: *Pleurotus florida, bioaccumulation, heavy metals, polycyclic aromatic hydrocarbons, oyster mushroom, substrates.*

INTRODUCTION

Pleurotus. *Pleurotus* species are highly cultivated edible mushrooms accounting for about 25% of global mushroom production.(Galappaththi *et al*., 2021). They are characterized by their shelf-like fan-shape caps and white, wood-decaying nature. Major cultivated species include *Pleurotus oestreatus, Pleurotus florida* and *Pleurotus pulmunarius,* which are valued for their high nutritional content, such as protein and fiber, as well as their medicinal properties, including antioxidants and anti-inflammatory effects. (Raman *et al*., 2021). Pleurotus species are known for their rapid growth and their ability to convert inexpensive Agricultural wastes (substrates) such as sawdust and straw into edible biomas. Due to these characteristics, it's considered popular, economic and environmental adaptable. (Sharma *et al*.,2019). Mushrooms generally are known for their strong bioaccumulation ability, meaning they can absorb and concentrate various substance from their growth substrates (Zhang *et al*., 2024). Due to their well-developed mycelial network and enzymatic system, Pleurotus species can accumulate heavy metals, hydrocarbons and other environmental pollutants present in contaminated soil or Agricultural wastes (substrates). This property makes the important in bioremediation studies (Liu *et al.*, 2024). However, the bioaccumulation of toxic substance in the fruiting bodies may also pose potential health risk if contaminated mushrooms are consumed, making monitoring and substrates selection essentials in mushroom cultivation (Siric *et a*l., 2022; Kumar *et al*., 2023).

Materials and Methods

The experiment was carried out at the Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Umudike is located in Umuahia South Municipality in Abia State, Nigeria. The coordinates for Umudike are 5.5011°N, 7.5380°E. The time zone is Africa/Lagos. (Nwankwo and Igboekwe, 2011). The spawn of *Pleurotus florida* was sourced from the Department of Biological Sciences at the Federal University of Technology, Owerri, Nigeria (FUTO). Substrates (sugarcane bagasse, saw dust, rice straw and *Andropogon* straw) were sourced from rural farm areas in Abia and Ebonyi State. The substrates were chopped into smaller pieces to increase the surface area, facilitating faster colonization by the mushroom mycelium. Two hundred grams (200g) of each substrate was measured at five different sets. Each substrate was moistened to achieve an optimal moisture content of approximately 60-65% before pasteurization. (Dewanu *et al.,* 2018; Akcay *et al*., 2023; Magana-Amaya and Shimizu, 2025).
The engine oil polluted soil was collected within 1 to 10cm of soil, mechanic Village Umuahia, Abia State. The soil was sieved to remove debris, the polluted soil was measured in four different levels (50g, 100g, 150g and 200g) respectively for each substrate. The polluted soils were sterilized, allowed to cool and placed under 200g of pasteurized substrate, before they were aseptically inoculated with 100g of pure spawn culture of *P. florida* according to Oyetayo, 2019 and Adebayo *et al*., 2021. The control was mushroom grown on each substrate respectively without the polluted soil. Once the substrates were cooled, Inoculation was done under sterile condition to minimize the risk of contamination. To achieve this, the lab was cleaned and disinfected, all equipment including knives; gloves and containers were sterilized using alcohol. The sterilized substrates were placed in clean plastic containers; 100g of spawn was evenly distributed aseptically throughout the substrates respectively. After which the substrates and spawn were mixed thoroughly to ensure even distribution of the mycelium. The containers were sealed to maintain humidity and prevent contamination. Small holes were made on the plastic buckets to allow gas exchange. The inoculated substrates were placed in an incubation room with a controlled temperature (around 25-30°C) and humidity (approximately 80-90%) to promote mycelia growth. The incubation lasted for 10-14 days, during which the mycelium colonized the substrate (Oyetayo, 2019 and Adebayo *et al*., 2021). The data collected the fresh weight, the total number of fruiting bodies, the stipe length, the cap diameter, and the dry weight (g): the dry weight was recorded after the harvest. The experiment was set up in a Completely Randomized Design (CRD) with four treatments replicated five times. The data collected were subjected to Analysis of Variance (ANOVA), and means were separated using Tukey's HSD at $P \leq 0.05$ (Olayanju *et al.*, 2020). The polluted soil was analyzed to ascertain the Polycyclic aromatic hydrocarbons (PAHs) and heavy metals concentration. Samples of *P. florida* harvested from different substrates were also analyzed to assess the micro accumulation of Polycyclic aromatic hydrocarbons (PAHs) and heavy metals by the mushroom, comparing it with the PAHs and heavy metals concentration of the engine oil polluted soil (Dewi *et al.*, 2019; Rai *et al*., 2021).

Results and Discussion

Table 1: Substrates used

BOTANICAL NAME	COMMON NAME
Oryza sativa	Rice straw
Saccharum officinarum	Sugar cane bagasse
Andropogun gayanus	Gamba grass
Ace specie	Marple tree

Table 2: Heavy metals accumulation potential of *Pleurotus florida* grown on Engine Oil

	Znppm	Crppm	Asppm	Cdppm	Cuppm
ADPG	$0.22^{e} \pm 0.00$	$0.16^{e} \pm 0.00$	$0.19^{e} \pm 0.00$	$0.09^{d} \pm 0.00$	$0.01^{a} \pm 0.00$
SBS	$0.08^{c} \pm 0.00$	$0.12^{d} \pm 0.00$	$0.12^{d} \pm 0.00$	$0.18^{e} \pm 0.00$	$0.01^{a} \pm 0.00$
SDST	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.0^{a} \pm 0.00$
RST	$0.11^{d} \pm 0.00$	$0.04^{b} \pm 0.00$	$0.07^{c} \pm 0.00$	$0.04^{b} \pm 0.00$	$0.00^{a} \pm 0.00$
MUPS	$0.03^{b} \pm 0.00$	$0.07^{c} \pm 0.00$	$0.04^{b} \pm 0.00$	$0.05^{c} \pm 0.00$	$0.00^{a} \pm 0.00$

Means followed by the same alphabet within row are not significantly different by Tukeys HSD at $(P \leq 0.05)$
Where MUPS = Mushroom grown on unpolluted soil (control), SBS = Sugarcan Baggerse, RST = Rice Straw, SDST = Sawdust and ADPG = Andropogun.
Znppm: Zinc part per million, Crppm: Chromium part per millions, As ppm: Aresmic, Cdppm: Cadmium, Cuppm: copper

The effect of different heavy metals accumulation by *Pleurotus florida* is shown in

Table 2. Five (5) heavy metals were detected in the engine oil polluted soil before mushroom cultivation. Among them were Zinc (Zn), Chromium (Cr), Arsenic (As), Cadmium (Cd) and Copper (Cu). This result showed a significant ($P \leq 0.05$) traces of Zinc, chromium, Arsenic and Cadmium accumulation in the mushroom fruiting bodies harvested from *Andropogun* straw, sugar bagasse, Rice straw and control. while there was no significant different of copper accumulation in the mushroom fruiting bodies across the substrates and control. Also, there was no significant different of zinc, chromium, Arsenic, Cadmium and Copper accumulation in the mushroom fruiting bodies grown on sawdust. This makes sawdust a more excellent substrates for commercial mushroom farmers (growers).

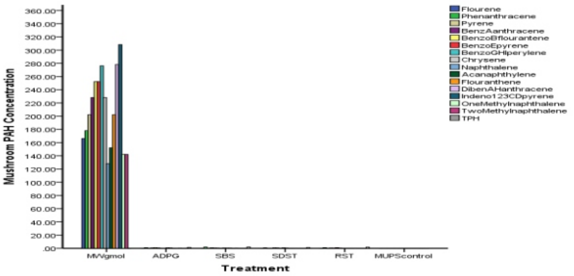

Plate1: Andropogun

Plate 2: sugarbagase

Plate 3: Rice straw

Plate 4: Sawdust

Fig 1: PAHS accumulation in the mushroom fruiting bodies

Figure 1 showed significant traces of PAHs accumulation in the mushroom fruiting bodies across the substrates. The highest amount of PAHs (Phenanthracene) accumulation was found among mushrooms harvested from sugar bagasse (1.91d ± 0.00) while the least PAHs (Flourene) accumulation was obtained from saw dust (0.17b ± 0.00), with no trace of PAHs concentration in the control. This observation was clearly shown across the control which indicates no trace of PAHs in the mushroom fruiting bodies.

Discussion

The presence of heavy metals such as zinc (Zn), chromium (Cr), arsenic (As), cadmium (Cd), and copper (Cu) in the engine-oil polluted soil prior to mushroom cultivation indicates contamination of the soil by petroleum products. Spent engine oil is known to contain different toxic metals released from engine wear, additives, and fuel combustion, which accumulate in soil and become available for uptake by microorganisms and plants. Studies have reported that soils contaminated with petroleum products often contain elevated concentrations of heavy metals that may be transferred into organisms growing on such substrates (Ayangbenro and Babalola, 2020; Adeleke *et al.,* 2021). The significant accumulation ($P \leq 0.05$) of Zn, Cr, As, and Cd in the fruiting bodies of *Pleurotus florida* cultivated on substrates such as *Andropogon gayanus* straw, sugarcane bagasse, and rice straw indicates the strong bioaccumulation potential of oyster mushrooms. Mushrooms belonging to the genus Pleurotus possess an extensive mycelial network capable of absorbing and concentrating heavy metals from contaminated substrates. Recent research has shown that oyster mushrooms can accumulate metals such as cadmium, chromium, and zinc from polluted environments due to their efficient nutrient absorption mechanisms and large surface area of fungal hyphae (Ayangbenro and Babalola, 2020; Usman *et al.,* 2022). This characteristic has led to the increasing use of Pleurotus species in mycoremediation, where fungi are used to remove or reduce pollutants from contaminated soils.

However, the absence of significant differences in copper accumulation across the substrates and control suggests that copper uptake by the mushroom may be relatively regulated. Copper is an essential micronutrient required for fungal enzymatic activities and metabolic processes. Therefore, fungi often maintain copper concentrations within physiological limits through regulatory mechanisms that control its uptake and storage (Sirik *et al.*, 2022). Interestingly, mushrooms cultivated on sawdust showed no significant accumulation of Zn, Cr, As, Cd, and Cu. This observation suggests that sawdust may reduce the bioavailability or absorption of heavy metals by the mushroom. Lignocellulosic materials such as sawdust are known to possess adsorptive properties that can bind or immobilize heavy metals, thereby limiting their transfer into fungal fruiting bodies. Consequently, sawdust is widely recommended as a safer and more suitable substrate for oyster mushroom cultivation, especially for commercial production where food safety is important (Hoa and Wang, 2020; Koutrotsios *et al.,* 2021). The result therefore indicates that sawdust may serve as a more appropriate substrate for mushroom farmers due to its lower risk of contaminant accumulation.

Regarding polycyclic aromatic hydrocarbons (PAHs), the results showed significant traces of PAHs in mushroom fruiting bodies grown on the different substrates. PAHs are common environmental pollutants produced from the incomplete combustion of petroleum products such as engine oil. The accumulation of PAHs in the fruiting bodies of *Pleurotus florida* indicates that these compounds can be absorbed from contaminated growth substrates. White-rot fungi such as *Pleurotus* species possess ligninolytic enzymes, including laccases and peroxidases, which enable them to degrade or transform complex aromatic hydrocarbons such as PAHs (Haritash *et al.,* 2020; Koutrotsios *et al.,* 2021). The highest concentration of phenanthrene observed in mushrooms cultivated on sugarcane bagasse suggests that substrate composition can influence the availability and uptake of PAHs by fungal tissues. Agricultural residues such as bagasse may enhance the retention or mobility of hydrocarbon compounds, thereby increasing their accumulation in mushroom fruiting bodies. Conversely, the lowest concentration of fluorene observed in mushrooms grown on sawdust further supports the earlier observation that sawdust may limit the absorption of contaminants. This may be attributed to the lignocellulosic structure of sawdust, which can adsorb hydrocarbons and reduce their transfer into the mushroom tissues (Koutrotsios *et al*., 2021). The absence of PAHs in the control treatment indicates that contamination in the mushroom fruiting bodies likely originated from the polluted substrates rather than from the mushroom itself. This finding highlights the importance of substrate selection in mushroom cultivation, as contaminated substrates may lead to the accumulation of harmful compounds in edible mushrooms.

In conclusion, the results demonstrate that *Pleurotus florida* is capable of accumulating both heavy metals and PAHs from contaminated substrates. While this property makes the fungus useful for environmental remediation, it also raises concerns regarding the safety of mushrooms cultivated on polluted substrates. Therefore, the use of clean and uncontaminated substrates such as sawdust is recommended for safe commercial mushroom production.

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