Effect of *Pleurotus ostreatus* on the Polycyclic Aromatic Hydrocarbon and Heavy Metal Concentrations of Lubricating Oil-Amended Soil

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Abstract: Background**:** The need to develop an ecologically friendly and efficient approach of removing contaminants from polluted soil has been a major area of interest to save the environment. This study was conducted to investigate the effect of *Pleurotus ostreatus* on the polycyclic aromatic hydrocarbon (PAH) and heavy metal concentrations of lubricating oil-amended soil. The white-rot fungus pre-grown on saw dust was investigated for its potential to remediate polycyclic aromatic hydrocarbons (PAH) and accumulate heavy metals in 20 % (v/v) unused and used lubricating oil-amended soil after 30, 60 and 90 days of incubation at 28^0C. Results obtained revealed an overall reduction of 69.52 % and 85.59 % PAH in treatment 1 and treatment 2 accordingly. There was also an overall reduction in heavy metal concentrations of the different heavy metals analyzed. Lead(Pb) reduced by 91.92 % and 63.45 %, zinc(Zn) reduced by 67.11 % and 70.08 %, copper(Cu) reduced by 49.58 % and 62.01 % while iron(Fe) also reduced by 62.42 % and 64.07 % after 90 days of incubation with *P. ostreatus* for treatment 1 and treatment 2 respectively. The significant reduction in the concentration of PAH and heavy metals over the time of incubation with *Pleurotus ostreatus* indicates that effective mycoremediation could be achieved using *Pleurotus ostreatus* to restore an impacted soil.

Keywords: *Pleurotus ostreatus,* heavy metals, hydrocarbons, lubricating oil, contaminants, soil.

BACKGROUND

Hydrocarbon discharge by human activities has impacted negatively on the environment. Some of these consequences are felt by plants, animals, humans and microorganisms (Maduwuba *et al*., 2021a). These hydrocarbons are introduced into the environment during oil production, transportation, and accidental spill (Maduwuba *et al*., 2021b). Many industrial activities release hazardous substances into the environment. One type of petroleum derivative that has proven to be a common environmental pollutant is lubricating oil. This oil contains of alicyclics, alicyclics aromatics, steranes, saturates, hopanes, heavy metals, parraffins, and esters which are known to have carcinogenic and mutagenic effects (Reddy and Mathew, 2001; Maduwuba, 2022). Lubricating oil discharge into the environment is a challenge due to its frequency and volume of discharge into the ecosystem through numerous routes. Some of these routes of discharge are; heavy duty trucks, operational equipment, generators, automobile, heavy-duty engines, gears, motorcycles and hydraulics (Abioye *et al*., 2010).

Pleurotus ostreatus is an edible fungus commonly known as the oyster mushroom. The fungus was named *Pleurotus,* which means side ear in Latin, and the oyster name (*ostreatus)* was based on its resemblance to shells of oyster as described by a German mycologist known as Paul Kummer (Kapahi and Sachdeva, 2017a). *P. ostreatus* is incapable of synthesizing its nutrient using CO_2, due to absence of chlorophyll. Meanwhile, it is highly saprophytic, growing on decaying organic matter, where organic nutrients are digested by secreting extracellular enzymes, which digest the components before assimilation (Bamiro and Osibanjo, 2004; Ogbo, 2009; Isikhuemhen *et al.,* 2003).

P. ostreatus is widely distributed in nature, especially in Britain, Ireland, Asia, America, Europe, and Africa. Its proliferation is strongly enhanced by availability of moisture. The fungus is widely optimized in the food and pharmaceutical industries.

It also plays a vital role in environmental bioremediation (Adenipekun *et al*., 2011a). Heavy metals such as mercury, lead, chromium, cadmium etc; are also introduced into the soil through effluent discharge and run-off (Lale *et al*., 2014; Maduwuba, 2022). This metals accumulate in the soil, and are either deposited into water bodies or absorbed and accumulated by plants. In each case, humans are exposed to the heavy metals when such crops, aquatic animals and water are consumed. Also, the biomagnification of these heavy metals poses threat to the overall cellular activities of cells (Okeola *et al*., 2011).

The polycyclic aromatic hydrocarbons (PAH) which is a major component of lubricating oil, are produced as a result of incomplete combustion and when introduced into the soil increases contamination rate (Ogbeh *et al.,* 2018). The hydrocarbon and heavy metals disrupt soil fertility by limiting nutrient absorption and plants' growth, elimination of valuable soil microorganisms, and reduction in micro, macro and mycoflora in the soil (Baldrian *et al.,* 2000).

The use of chemicals and chemical related products to tackle the high pollution effect of PAH and heavy metals from lubricating oil in the environment has further impacted negatively on the ecosystem and as such making it imperative to develop an ecologically friendly approach to restore the environment. Hence, the use of *Pleurotus ostreatus* as a cost effective and ecologically friendly approach in combating environmental pollution (Kapahi and Sachdeva, 2017b; Baldrian *et al.,* 2000; Adenipekun and Isikhuemhen, 2008). Therefore this study is aimed at monitoring the effect of *Pleurotus ostreatus* on the polycyclic aromatic hydrocarbon and heavy metal concentrations in lubricating oil amended soil. The result obtained from this study would provide a potent mycoremediation process which can be applied for environmental restoration.

MATERIALS AND METHODS

Sample Source

The soil sample used for this study is garden soil collected from the Agricultural Demonstration Farm, University of Port Harcourt using the method of Adenipekun *et al* (2011). The co-ordinates of the soil sampling points were determined using the Global Positioning System (GPS) as; $4^{0}54^{1}42^{11}$N and $6^{0}54^{1}32^{11}$E for point 1, $4^{0}55^{1}44^{11}$N and $6^{0}55^{1}52^{11}$E. This area has not experienced any incidence of hydrocarbon pollution. The soil collected is a sandy-loam soil. Saw dust was used as the fungal substrate, and this was collected from the wood saw mill, Timber market at Rumuosi, Port Harcourt. The lubricating oil product for this study is Mobil XPH. Waste oil was collected from a car engine at a mechanic workshop after three months of usage. All materials were transported to the environmental microbiology laboratory, Department of Microbiology, University of Port Harcourt Nigeria.

Pleurotus ostreatus Cultivation and Identification

The fruiting body (mushroom) of the fungus was grown at the Agricultural Demonstration Farm, University of Port Harcourt. The fruiting bodies were subjected to macroscopic and microscopic examination. The macroscopic examination included texture, colour, shape and smell characteristics done by physical observation. The microscopic examination was done using a thin section of the fruiting body soaked in distilled water viewed on a light microscope to observe for spores and basidia. The spore prints were also observed for their colour (Weber and Webster, 2006). The fungi were identified as *P. ostreatus* based on its macroscopic and microscopic morphological characteristics by the mycology unit of the department of plant science and biotechnology, University of Port Harcourt, Nigeria.

Mycelium Production of *Pleurotus ostreatus*

Tissue culture procedure was used to produce the fungal mycelium. The medium glucose malt extract agar (glucose $10gL^{-1}$, malt extract $30gL^{-1}$ and agar $15gL^{-1}$) was used according to the method of Weber and Webster (2006). A sterile scapel was used to cut the stalk of the fruiting body to expose the inner section of the fruiting body (2mm x 2mm) lengthwise. This was aseptically placed at the center of the surface of the medium used and sealed with a para-film before incubating for 6 – 7 days in the dark. The mycelium obtained was further sub-cultured in a glucose malt extract agar plate to obtain a pure culture. The mycelium was also observed for their characteristics growth pattern and other cultural characteristics on the medium according to Baldrian *et al (*2000).

Twenty-five grams (dry wt. equivalent) of sawdust was weighed into ten different 250 ml conical flasks moistened with 50 % distilled water (v/w) and sterilized at 121^{o}C, 15psi for 15 minutes. After sterilization, the conical flasks were allowed to cool, then inoculated with two to three agar plugs of growing mycelium of *Pleurotus ostreatus* using a 7mm sterile cork borer. The conical flasks were covered and incubated at room temperature (28^{0}C) for 14 days for the mycelium of the fungus to properly colonize the saw dust.

Soil Processing and Contamination

The method of Baldrian *et al.* (2000) was also used. The soil collected was thoroughly homogenized and air-dried in sterile laboratory trays. The air-dried soil was passed through 2mm soil mesh to remove bigger particles and plant materials in it.

One hundred and fifty gram (150g) of garden soil was weighed into 500ml jam bottles and moistened with 10 % distilled water (w/v). The soil in each bottle was mixed thoroughly then sterilized and allowed to cool. Twenty-five percent (25%) concentration (w/w) of the lubricating oil was spiked into each of the moistened soil in the glass bottles and fifteen gram (15 g) of the ramified spawn of *Pleurotus ostreatus* was aseptically laid over the contaminated soil in each glass bottle. The set ups were incubated and monitored at room temperature (28^{o}C) for 90 days.

Experimental Design

This study followed the pre and post-experimental study pattern. The experimental setup consists of ten (10) experimental units separated into two (2) groups with two controls for the used and unused lubricating oil samples, respectively. Details of the experimental setups are shown in Table 1.

Table 1: The different experimental setups for treatment with *Pleurotus ostreatus*

TREATMENTS
Soil + engine oil + sawdust (treatment control at day 1)
Soil + engine oil + sawdust + *P.ostreatus* (treatment after 30 days)
Soil + engine oil + sawdust + *P.ostreatus* (treatment after 60 days)
Soil + engine oil + sawdust + *P.ostreatus* (treatment after 90 days)

Gas Chromatographic Analysis.

The procedure as described by Hu *et al.* (2014), method 3510C with slight modification was employed. This was done by adding 10 ml of dichloromethane to 2 g of the soil sample. The mixture was stirred thoroughly and allowed to settle then filtered through an extraction column. The extract was then concentrated to 2 ml using evaporation and transferred into labelled glass vials with Teflon rubber crimp caps. 1 µl of the concentrated sample was injected by means of a hypodermic syringe through a rubber septum into the column where its separated components are detected by the gas chromatography-flame ionization detector HP5890 Series II (GC-FID). This method was carried out for each of the

treatment options at days 1, 30, 60 and 90 respectively.

Heavy Metal Analysis

The reduction in heavy metal concentrations for Iron (Fe), zinc (Zn), lead (Pb) and copper (Cu) were also monitored using the atomic absorption spectrophotometer HACH DR 2401 USA. The method as described by Singh (1980) and Maduwuba *et al.* (2021b) was employed. 10 g each of air-dried soil samples were digested with 0.2 M nitric acid (HNO_3^-) solution for 60 minutes in a flask over high heat. Care was taken to avoid the acid from drying out by adding extra acid solution periodically until digestion was completed. The digests were then filtered separately through a Whatman filter paper and the volume made up to 100 ml by flushing the sample with distilled water. The different filtrates were then analyzed using the atomic absorption spectrophotometer (AAS) whose setting were done following the maufacturer's guidelines and calibrated using the analytical grade metal standard stock solutions (1 mg/dm^3) in triplicates.

RESULTS

Base-Line Result

The morphological characterization of *Pleurotus ostreatus* used for this study and the baseline physico-chemical characterization of the soil only (sample A), the soil mixed with unused oil (sample B1) and soil mixed with the used oil (sample B2) were carried out. The results are presented in tables 2 and 3.

Table 2: Morphological characteristics of the white rot fungus used for the study

Examinations	Observations
Macroscopic	
Pileus	5 – 10 cm broad, smooth surface, white to grey colour, oyster shaped margin and convex to flabellate.
Lamella	Extended decurrent crowded, white to cream colour, 1.0 – 3.0 cm diameter with solid lamellae.
Context and Stipe	White thin to thick flesh body, short, radially porous with mild scent of anise. Eccentric stipe of 2.3 – 4.2 cm in length
Mycelia forms	Aerial growth with edge forms, concentric circular with dense and light growth on plates.
Microscopic	
Spores	White spores, 3.3 – 4.2 µm, fine, elliptical and non amyloid.
Basidia	Slender clavate and tetrasporic.

The organism was identified as *Pleurotus ostreatus*.

Table 3: Soil Composition and physico-chemical characteristics of pre-treatment controls used for baseline study

PARAMETER	SAMPLE		
	A	B1	B2
Sand (%)	72.87	-	-
Silt (%)	19.92	-	-
Clay (%)	7.21	-	-
Polycyclic Aromatic Hydrocarbon (mg/kg)	-	4.518	9.273
pH	6.57	6.40	6.59
Zinc (mg/kg)	0.63	11.68	12.62
Lead (mg/kg)	2.209	28.42	44.18
Copper (mg/kg)	0.296	7.56	5.92
Iron (mg/kg)	23.197	463.04	463.76

Key: A: soil only; B1: soil spiked with unused lubrication oil; B2: soil spiked with used lubrication oil.

Polycyclic Aromatic Hydrocarbon (PAH)

The changes in the polycyclic aromatic hydrocarbon (PAH) concentrations showed a reduction of PAH from 2.92±0.037 mg/kg at day 1 (control 1) to 2.09±0.024 mg/kg at day 30, 1.42±0.075 mg/kg at day 60 and 0.89±0.022 mg/kg at day 90 which is equal to 28.42 %, 51.37 % and 69.52% reductions, respectively for treatment 1 while in treatment 2, PAH reduced from 7.085±0.011 mg/kg, (control 2) to 5.142±0.138 mg/kg at day 30, 3.68±0.139 mg/kg at day 60 and 1.021±0.058 mg/kg at day 90 which is equal to 27.42 %, 48.66 % and 85.59 % reductions, accordingly over the treatment period. Control 2 had the highest PAH concentration of 7.085±0.011 mg/kg while control1 had a PAH concentration of 2.92±0.037 mg/kg. The lowest PAH concentration of 0.89±0.022 mg/kg was obtained in treatment 1 (unused lubrication oil) after 90 days. The result is shown in figure 1.

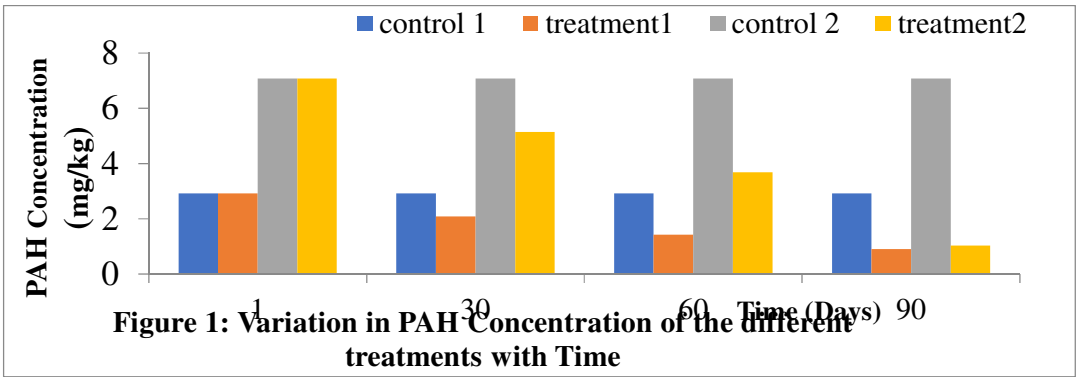

Figure 1: Variation in PAH Concentration of the different treatments with Time

Heavy Metal Concentration

Lead concentration was reduced over the treatment period with *P. ostreatus*. In treatment 1, lead concentration reduced from 23.51±0.008 mg/kg (control 1) to 18.95±0.043 mg/kg at day 30, 4.20±0.216 mg/kg at day 60 and 1.90±0.00 mg/kg at day 90 showing percentage loss of 19.40 %, 82.14 % and 91.92 % respectively while in treatment 2, there was reduction from 33.0±2.16 mg/kg (control 2) to 29.45±0.00 mg/kg at day 30, 22.09±0.033 mg/kg at day 60 and 12.06±0.016 mg/kg at day 90.

Treatment 1 at day 90 had the highest percentage loss of 91.92% while treatment 2 at day 30 had the lowest percentage loss. The lead concentration of control 2 was higher than that of control 1. The result is shown in figure 2.

There was a reduction in zinc concentration over the period of treatment. In treatment 1, zinc content reduced from 9.12±0.014 mg/kg (control 1) to 7.79±0.032 mg/kg at day 30, 6.13±0.029 mg/kg at day 60 and 3.0±0.041 mg/kg at day 90 resulting in percentage loss of 14.58%, 32.79% and 67.11%, respectively. In treatment 2, zinc content reduced from 9.86±0.05 mg/kg (control 2) to 8.413±0.066 mg/kg at day 30, 6.13±0.00 mg/kg at day 60 and 2.95±0.040 mg/kg at day 90 which is equal to a percentage loss of 14.68%, 36.0% and 70.08%, respectively over the treatment period. Control 2 had a zinc concentration of 9.86±0.05 mg/kg which was higher than that of control 1 that had a concentration of 9.12±0.014 mg/kg. The result is shown in figure 3.

The copper concentration also reduced during the treatment period. Treatment I reduced from 5.91±0.083 mg/kg (control 1) to 5.04±0.00 mg/kg at day 30, 4.01±0.106 mg/kg at day 60 and 2.98±0.12 mg/kg at day 90 resulting in a percentage loss of 14.72 %, 32.15 % and 49.58 %, respectively at the different points of sampling. Treatment 2 reduced from 4.87±0.00 mg/kg (control 2) to 3.95±0.021 mg/kg at day 30, 3.0±0.213 mg/kg at day 60 and 1.85±0.00 mg/kg at day 90 resulting in a percentage loss of 18.89 %, 38.39 % and 62.01 %, respectively at different points of sampling. Control 1 had a concentration of 5.91±0.083 mg/kg which was higher than that of control 2 that gave 4.87±0.00 mg/kg. The highest percentage loss of 62.01 % occurred in treatment 2 at day 90. The result is shown in figure 4.

The iron concentration of the different samples was very high compared to the other heavy metals monitored. There was reduction in the concentration of iron as mycoremediation progressed. In treatment 1, iron content reduced from 400.23±0.209 mg/kg (control 1) to 308.69±1.010 mg/kg at day 30, 229.80±3.84 mg/kg at day 60 and 150.40±1.158 mg/kg at day 90 showing percentage loss of 22.87 %, 42.54 % and 62.42 %, respectively. Treatment 2 reduced from 405.80±0.903 mg/kg (control 2) to 309.17±0.912 mg/kg at day 30, 230.80±0.025 mg/kg at day 60 and 145.80±0.980 mg/kg at day 90 resulting in percentage loss of 23.81 %, 43.12 % and 64.07 %, respectively during the treatment period. The result is shown in figure 5.

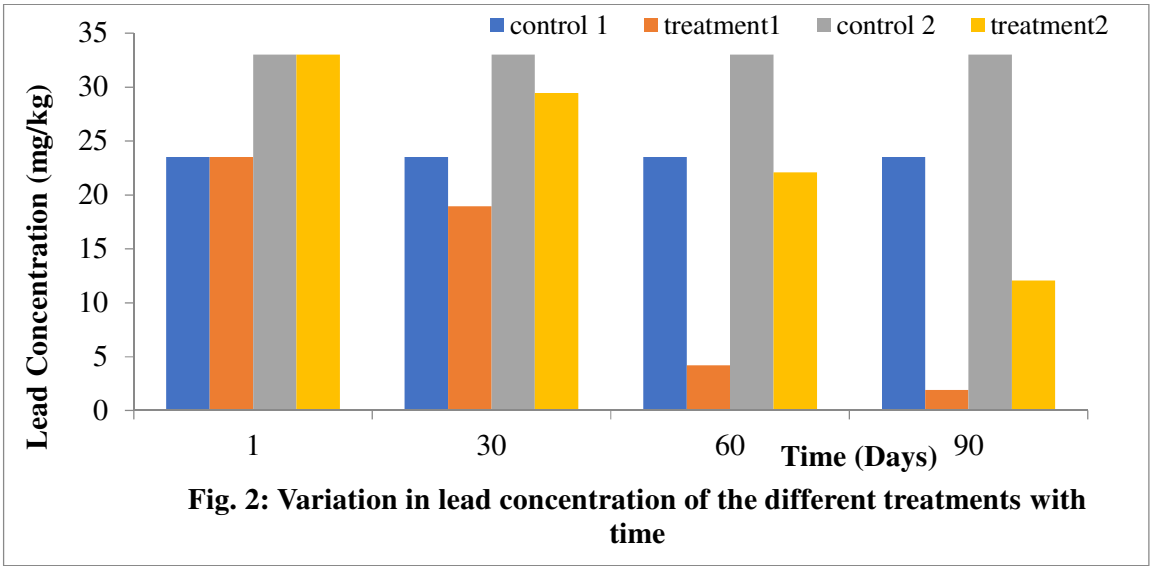

Fig. 2: Variation in lead concentration of the different treatments with time

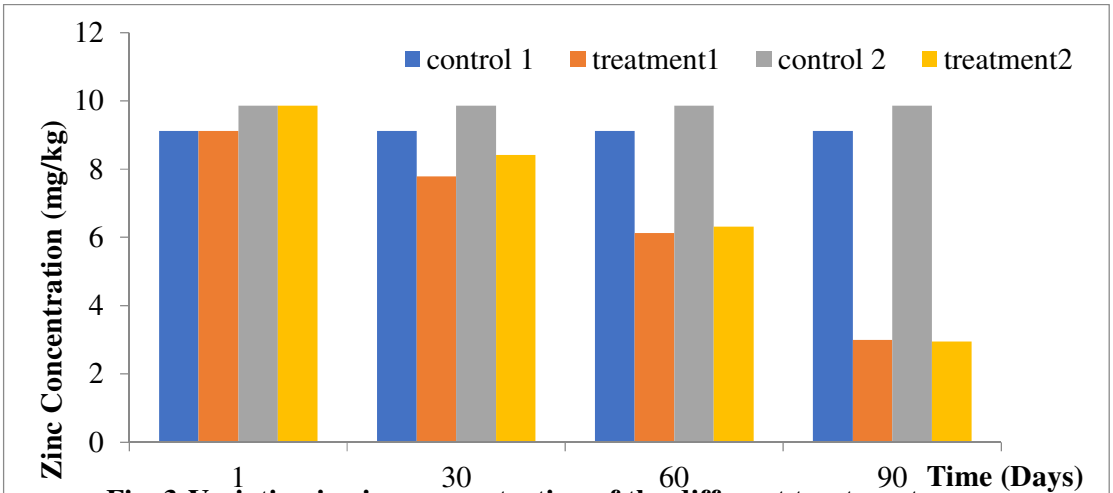

Fig. 3:Variation in zinc concentration of the different treatments with time

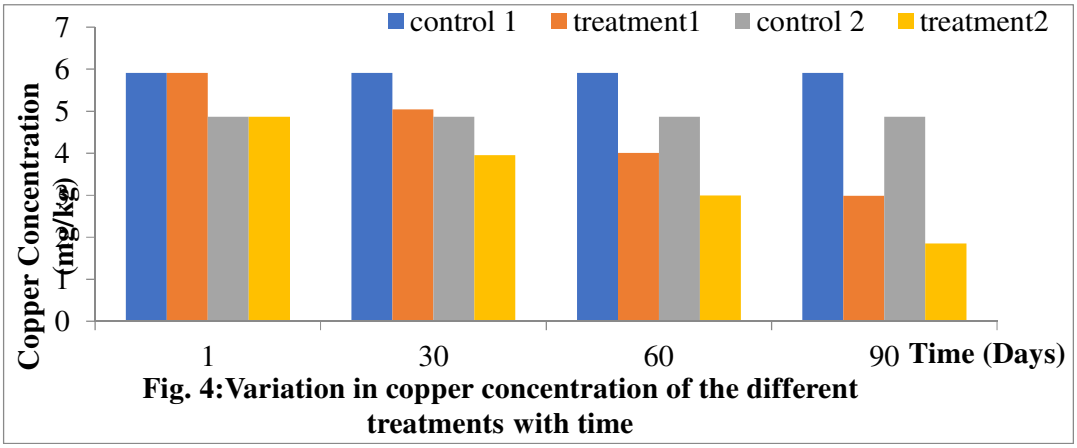

Fig. 4:Variation in copper concentration of the different treatments with time

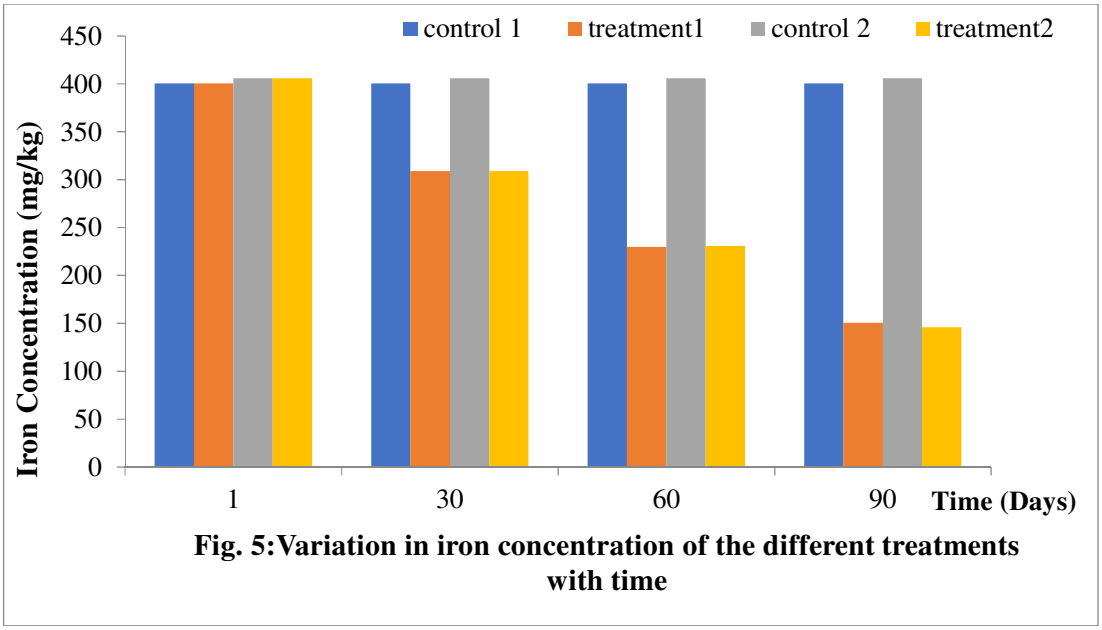

Fig. 5:Variation in iron concentration of the different treatments with time

DISCUSSION

The morphological and microscopic characterization of *P. ostreatus* revealed structures such as pilus, lamella, stipe, mycelium, spores, and basidia which confirms the fungal and saprophytic nature of *P. ostreatus*. This is in agreement with the study conducted by Kapahi and Sachdeva (2017a); Adenipekun *et al*. (2011a) and Adenipekun and Omoneyi (2008). The physicochemical analysis of the contaminated soil revealed the presence of polycyclic aromatic hydrocarbon (PAH) proving that the soil samples were contaminated by the deposits of lubrication oil. Studies have shown that most of the heavy metals present in the soil are vital for optimum functioning of soil microbes (Ali *et al.,* 2020) and plant species except lead which is highly toxic at very low concentrations (Ekperusi *et al.,* 2016). However, plants grown in such areas are likely to experience nutrient deprivation, toxification, and stunted growth with increase in heavy metal concentration. The heavy metals detected in this study include zinc, lead, copper, and iron. The heavy metals detected confirmed the study conducted by Yi *et al*. (2021); Kapahi and Sachdeva (2017a) which states that PAH in the soil enhances heavy metal accumulation. Heavy metals are highly toxic to living organisms even at moderate concentrations (Adenipekun *et al*., 2011b; Okeola *et al*., 2011). Soil microorganisms are inhibited by these metals, and when they are absorbed by consumable plants, biomagnification occurs in man, which poses a serious threat to body physiology (Kapahi and Sachdeva, 2017b). The ability of *P. ostreatus* to grow in the presence of lubrication oil contamination through their mycelial colonization conforms to the work of Aust and Swanner (2003) who reported that *P. osreatus* can withstand toxic levels of most organopollutants. Adenipekun *et al.* (2011a) also demonstrated that fungi are able to grow optimally in the presence of harmful contaminants and have the ability to detoxify such contaminants.

In this study, it was observed that there was a reduction in the PAH concentrations. This is a clear indication that *P. ostreatus* was able to utilize the hydrocarbons in the lubrication oil found in the soil for its metabolic activities and growth. It also indicated that mycoremediation occurred and this was confirmed by the work of Gardy (1985) who reported that mycoremediation involves transformation of complex or simple chemical compounds into non-hazardous forms by fungi resulting in materials of higher nutritive value or simply reducing the final bulk of the product. This could be attributed to the ability of *P. ostreatus* to secrete lignin degrading enzymes such as laccase, lignin peroxidase and manganese peroxidase utilizing them in degradation function. These enzymes have increased its ability to proliferate in the presence of toxic chemical compounds, which could inhibit the growth of other organisms and at the same time help the fungi to detoxify and utilize the chemical contaminants as energy and carbon source (Adenipekun *et al*., 2011b; Yi *et al.,* 2021). An interesting feature observed in mycoremediation is the ability of the fungus to transform the toxic chemical components to eco-friendly products.

The concentrations of heavy metals monitored were slightly below the Department of Petroleum Resources [DPR], (2002) stipulated target limits for micropollutants in soil except for iron concentration which was high in the soil. The high concentration of iron recorded agrees with the study of Osu *et al.* (2021) and Adesina and Adelasoye (2014) which recorded high iron concentrations in hydrocarbon polluted soils. This may be attributed to a high degree of pollution by petroleum-derived hydrocarbons (Tobin, 2001). The high iron (Fe) concentration in the sample could also be attributed to the function of lubrication oil as a lubricant to maintain movement and reduce friction in

the moving parts of machines. The spatial difference in the heavy metal concentrations observed in the different experimental setups during treatment with *P. ostreatus* indicates that bioaccumulation of heavy metals must have been carried out by the white-rot fungus. This finding is similar to the observation of Gilbertson (2004) that wood decaying fungi also have the ability to accumulate heavy metals. Kalac *et al.* (2006) and Gaddy (2013) have also used fungi for the treatment of heavy metal-containing effluent due to their ability to accumulate metals from their external environment. The increase in heavy metals in the soil as a result of the lubrication oil contamination was also confirmed from the baseline study. These heavy metals have been observed to be one of the most important factors that affect the microbial compositions of the environment where they are present (Zeng *et al.,* 2020; Zeng *et al.,* 2019). They cause an alteration or shift in the bacterial community structure (Xiao *et al.,* 2019).

The reduction in the concentration of heavy metals observed in this study could be ascribed to adsorbing potentials of *P. ostreatus* (Hassan *et al*., 2021; Huang *et al*., 2021; Yi *et al.,* 2021). The large surface area of the fungus enables it to trap heavy metals unto its surface for bioconversion, facilitated by its gill. This observation confirmed the study of several researchers (Kapahi and Sachdeva, 2017a; Okeola *et al.,* 2011; Yi *et al.,* 2021) who investigated the mycoremediation potential of *P. ostreatus.*

CONCLUSION

Heavy metals and polycyclic aromatic hydrocarbons (PAH) are major contaminants in the soil, which could emanate from crude oil products and industrial effluents. Contaminated soil interferes with crop production due to nutrient deprivation and toxification. The fruiting body of *Pleurotus ostreatus* is capable of neutralizing the effects of heavy metals and PAH in the soil, as revealed in drastic reduction in the concentration of the contaminants after treatment. It can therefore be stated that *Pleurotus ostreatus* can be used as an effective tool for bioremediation haven proved to be efficient, less expensive, adaptable and environmentally friendly for the recovery of impacted sites.

List of Abbreviations

P. ostreatus: Pleurotus ostreatus
PAH: Polycyclic Aromatic Hydrocarbon
DPR: Department of Petroleum Resources
EGASPIN: Nigeria environmental guideline and standards for the petroleum industry in Nigeria

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