

Mycoremediation of Spent Lubricating Oil Contaminated Soil Using *Pleurotus ostreatus***Maduwuba, M. C.***

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Abstract: This study was conducted to evaluate the remediation capability of *Pleurotus ostreatus* on spent lubricating oil polluted soil. Twenty percent (v/w) lubricating oil was spiked into 100 g of garden soil, inoculated with *P. ostreatus* pre-grown with sawdust as a substrate. The changes in pH, moisture content, total petroleum hydrocarbon, percentage total organic carbon, nitrate and phosphate concentrations were monitored biweekly for 84 days. Results obtained showed an increase of pH from 6.21 to 6.79. Total petroleum hydrocarbon concentration reduced by 72.5 % from 2892.10 mg/kg at day 1 to 796.66 mg/kg at day 84. The percentage TOC increased by 73.3 % from 3.16 % to 5.467 % while there was decrease in moisture content from 24.81% to 10.80% (56.5 % reduction). There was 62 % decrease in the concentration of phosphate from 18.80 mg/kg to 7.14 mg/kg while 5.4 % decrease in nitrate concentration from 89.48 mg/kg to 84.68 mg/kg was observed. Analysis of variance (ANOVA) showed an overall significant difference between the concentration changes over time at 95% confidence interval. The results of this study proved the capability of *P. ostreatus* as an effective remediation tool for the recovery of hydrocarbon impacted soil.

Keywords: Mycoremediation, *P. ostreatus*, Spent, Lubricating, Oil.

INTRODUCTION

The release of industrial effluents into the environment contributes to hazardous contaminants that negatively affect the environment (Abdulkarim *et al.*, 2019). One of such substances observed to be common environmental pollutant is spent lubricating oil which is a viscous organic liquid, used to reduce friction between surfaces and for lubricating various combustion engines (Abdulyekeen *et al.*, 2016). The oil has the ability to improve fastening, cleanup, reduce the heat of engine parts and inhibit metal corrosion (Umana *et al.*, 2016). Lubricating oil consist mainly of saturates, monoaromatics, polyaromatics, hopanes, steranes, heavy metals, paraffins and esters (Abdulyekeen *et al.*, 2016). Additives such as amines, phenols, benzene, calcium, zinc, barium, magnesium, phosphorus, lead and sulphur can also be found in spent lubricating oil (Lale *et al.*, 2014).

The sources of used lubricating oil pollution include: used gear oils, engine oils, cutting oils, and hydraulics from automobiles, transmission oils, shock absorbers, refrigerators, generators and other heavy duty equipments (Baldrain *et al.*, 2000). As a result, a large amount of this spent lubricating oil is generated from the transportation,

construction and industrial sectors (Boughton and Horvath, 2004). Apart from hydrocarbons, dust, rust, detritus and metal particles are present in used lubricating oil because of its circulation within the engine (Reddy *et al.*, 2002).

The chemical composition of petroleum hydrocarbons is the main cause of its environmental pollution and ecological damage (Mohammadi-Sichani *et al.*, 2019). Lubricating oil can cause considerable damage to the environment mainly due to its high potential to cause serious water pollution (Boughton and Horvath, 2004). It also causes the pollution of soil sediments and water thus causing the death of resident organisms in such environments (Upshall *et al.*, 2003). Used lubricating oil also has a negative effect on human health (Thorsten, 2005). Also the additives contained in it can be toxic to the microbial flora and fauna in the soil and even plants and animals (Lale *et al.*, 2014). Lubricating oil contains heavy metals that can contribute to chronic (long term) hazards including carcinogens (Udom *et al.*, 2012). Metals of concern include lead, zinc, chromium, barium and arsenic (Irwin *et al.*, 2016; Adams *et al.*, 2014).

However, due to the high environmental pollution by lubricating oil derived hydrocarbons and its effects, it is important to

improve a cheap and effective means for the remediation of polluted systems (Adedokun and Ataga, 2013). The concept of mycoremediation as a biotechnological approach utilizes the metabolic ability of fungal organisms to recover hydrocarbon impacted systems (Watnabe, 2001; Adedokun and Ataga, 2013; Adenipekun and Isikhuemhen, 2003). This study however is aimed at examining the ability of the fungi *Pleurotus ostreatus* to degrade lubricating oil derived hydrocarbons in contaminated soil (Ejoh *et al.*, 2012).

Recently, the role of the white rot fungi (WRF) in the recovery of hydrocarbon polluted soil has generated considerable research interest (Bamiro and Osibanjo, 2004). The WRF are physiological group of fungus comprising of fungi that are capable of degrading complex hydrocarbons such as lignin (Ogbo *et al.*, 2006). The ability of this fungus to tolerate varying pH further improves their pollutant degrading potential (Verma and Madamwar, 2002). *Pleurotus ostreatus* is one of the groups of WRF with the ability of breaking down lignin and other xenobiotic substances (Stamets, 2000). They also secrete ligninolytic enzymes like lignin peroxidase, manganese peroxidase and laccases that enhance degradation (Ogbo 2009). Furthermore, mycoremediation using *Pleurotus ostreatus* could be low-technology, adaptive and environmentally friendly approach (Ejoh *et al.*, 2012).

MATERIALS AND METHODS

Soil Sampling

Sandy-loam soil was collected from Agricultural demonstration farm, University of Port Harcourt. This area has not recorded any incident of hydrocarbon pollution from lubricating oil prior to sample collection. The co-ordinates of the sampling points were determined using the Global Positioning System (GPS) as; 4°53'30"N; 6°54'30"E and 4°54'42"N; 6°55'50"E. The soil collected was Twenty millilitre of distilled water was added to 10 g of soil in a beaker and stirred manually then allowed to stand for about 30 minutes

homogenized, air-dried and passed through 2mm soil mesh. One hundred gram of soil was measured and put into each of the 500ml jam bottles, moistened with 10 ml distilled water then sterilized in an autoclave. Twenty millilitre of the lubricating oil was spiked into the moistened soil and mixed thoroughly according to the method of Adenipekun (2008) and Adenipekun *et al* (2011).

Mycelium Production of *Pleurotus ostreatus*

Tissue culture procedure was employed to produce *Pleurotus ostreatus* mycelium using glucose malt extract agar as described by Weber and Webster (2006). Mycelium inoculation and incubation was done by employing the modified procedure of Baldrain *et al.*, (2000). Twenty-five gram (dry weight equivalent) of saw dust was transferred into the ten different 250ml conical flasks moistened with distilled water, sealed with aluminium foil and sterilized at 121°C, 15psi for 15 minutes. The sterilized conical flasks were allowed to cool and then seeded with three agar plugs of growing mycelium of *Pleurotus ostreatus* each using a 7mm cork borer. The inoculated conical flasks were covered with cotton wool and aluminium foil and incubated for 14 days.

Mycoremediation experimental setup

Ten gram of ramified spawn of *Pleurotus ostreatus* was aseptically inoculated into the 20 % v/w of lubricating oil contaminated soil and incubated at 28°C. The setups were sampled every 14 days for a total of 84 days with the mixture of Soil + Lubrication oil + Saw dust (control) and also Soil + Lubrication oil + Saw dust + *Pleurotus ostreatus* (treatment) in duplicates (Adenipekun *et al.*, 2011).

Determination of Physico-chemical Parameters

The soil pH was monitored using the procedure adopted by Adenipekun and Isikhuemhen (2008).

and the pH was measured using a pH meter (HANNA H17343).

Moisture content was determined by gravimetric method as described by US EPA

(2004). Twenty gram of soil sample was placed in a moisture can and dried to a constant weight in an oven. The soil moisture

content was then calculated using the formula:

$$\text{Mc (\%)} = \frac{\text{Loss in wt}}{\text{Original wt}} \times 100$$

Where, Mc = moisture content (%) and wt = weight in gram

The total petroleum hydrocarbon (TPH) was determined using the cold extraction method as adopted by Bada *et al* (2019) with the gas chromatography model Hp5890 Series II equipped with FID agilent column. Extraction was done by adding 10ml of dichloromethane to 2g of the sample. The mixture was stirred and allowed to settle then filtered through an extraction bottles and concentrated to 2 ml by evaporation then placed into glass vials. One micro litre of the sample was injected through a rubber septum which is separated at the vapour partition between the helium gas and liquid phase. The fractions were detected along the column by the flame ionization detector (FID).

Total Organic Carbon (% TOC) was determined by the wet oxidation method of Schumacher (2002). One gram of the soil sample was put into a sterile conical flask with 5 ml potassium chromate solution and 7.5 ml concentrated sulphuric acid added, then heated for 15 minutes and allowed to cool. One hundred millilitre of distilled water was also added followed by 25ml of the solution and titrated with 0.2M ferrous ammonium sulphate using ferrion as indicator.

Nitrate (NO_3^-) concentration was determined using the brucine method (UNEP, 2004; 2011). One millilitre filtrate was measured into two different sterile test tubes. Two millilitre of concentrated H_2SO_4 was added

into the test tubes and mixed properly. The solution was allowed to cool then measured at 470nm using spectrophotometer (GBC918 PMT, China).

Phosphate concentration was analysed by ascorbic acid method as described by APHA (1992). The phosphate was extracted using 2.5% glacial acid. Fifty millilitre of each extract was then pipette into clean conical flasks and sterilized with $\text{K}_2\text{S}_2\text{O}_8$ and H_2SO_4 for 30 minutes at 121°C . Five millilitre of ammonium molybdate was added to the sterile mixture and reduced with stannous chloride to form molybdenum blue complex, then measured using spectrophotometer at 660nm.

Data analysis

The analytical data obtained within 84 days of sampling was subjected to one-way analysis of variance (ANOVA) using SPSS 20.0. The results obtained were considered statistically significant at 95 % confidence interval where $p < 0.05$.

RESULTS AND DISCUSSION

The properties of the unpolluted and polluted soil samples were analyzed and used as controls to compare the degradation process. The moisture content, organic carbon, nitrate, phosphate and total petroleum hydrocarbon for the polluted and unpolluted soil samples are as stated in table 1 below;

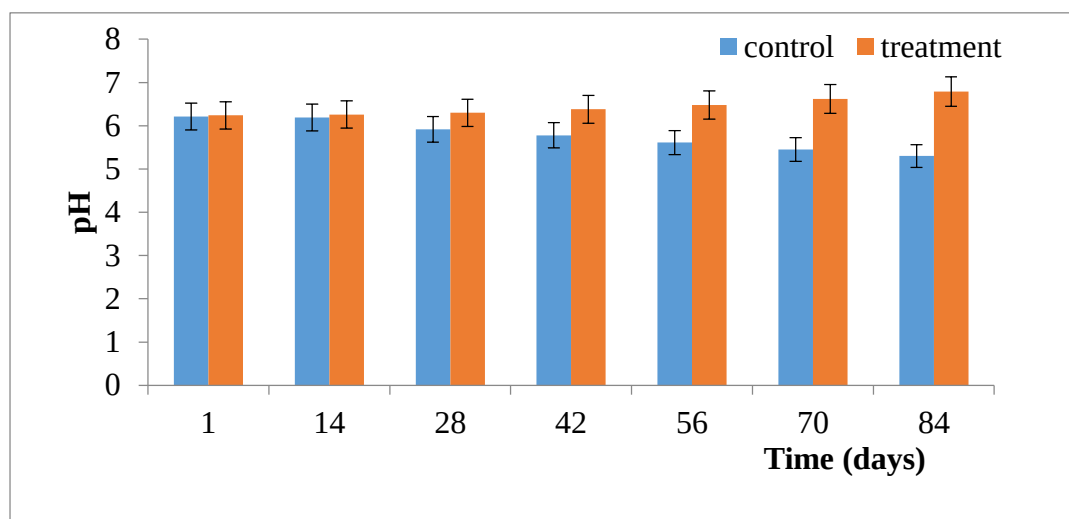
Table 1: Physico-chemical properties of spent lubricating oil contaminated and uncontaminated soil samples.

Parameters	Values	
	Before contaminated	After contaminated
pH	6.20±0.34	6.21±0.33
Moisture content (%)	18.0±2.09	24.81±0.43
Total Petroleum Hydrocarbon (mg/kg)	358±6.25	2892.1 ±0.00
Total Organic Carbon (%)	0.50±0.85	3.16±0.02
Nitrate (mg/kg)	10.05±0.27	89.48±0.04
Phosphate (mg/kg)	8.91±1.29	18.80±0.01

Figures = mean of duplicate values ± Standard deviation

The pH value of the control setups decreased while that of the treatment setups increased overtime. In the control, the pH from day 1, 14th, 28th, 42nd, 56th, 70th and 84th were 6.21, 6.19, 5.92, 5.78, 5.61, 5.45 and 5.30 at the termination of the experiment while that of the treatment were 6.21, 6.26, 6.30, 6.38, 6.48, 6.62 and 6.79 respectively (figure 1). There were slight differences between the values obtained at the different sampling periods of

5.78±0.325 for control and 6.44±0.189 for the treatment. The hydrogen ion concentration played an important part in the degradation process. The pH values recorded followed a consistent pattern and is in line with the work of Adenipekun and Omoruyi (2008) which showed that *Pleurotus* species carry out biodegradation activities at a slightly acidic to neutral pH.

**Fig 1: Changes in pH during treatment with *P. ostreatus***

Moisture content was observed to decrease as treatment progressed. Percentage moisture decreased from 24.81% to 23.47% for the control setup while the treatment setups reduced from 25.08% to 10.80% (figure 2). There was no significant difference between the results obtained from both the control and treatment setups. The mean value for the control was 24.139±0.43 % and treatment 17.94±4.76 %. The reduction in moisture

content in the soil can be attributed to the utilization of available moisture by the organism during its growth and degradation process according to Isikhuemhen *et al.*(2003). Also a similar work done by Adenipekun and Isikhuemhen (2008) attributed reduction in moisture content to the gradual evaporation of available moisture in the soil over a period of time.

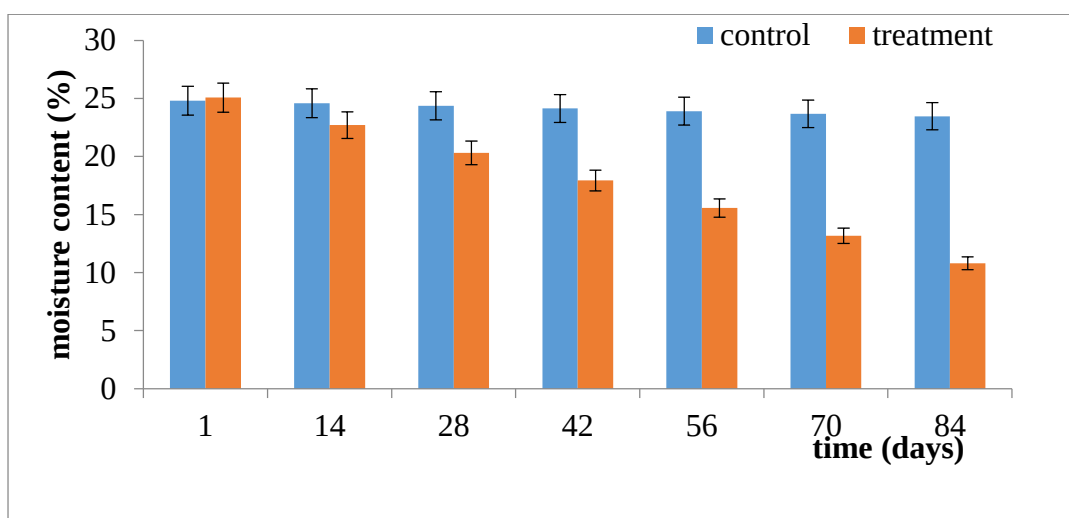


Fig 2: changes in moisture content during treatment with *P. ostreatus*

The change in total petroleum hydrocarbon (TPH) concentration was also monitored biweekly (14 days). The concentration of the control remained constant while there was an overall decrease in the concentration of the treatments. Concentration decreased from 2892.10 mg/kg to 796.662 mg/kg during the incubation period (figure 3) resulting to a percentage difference of 72.49 % after 84 days. Statistical analysis showed a significant difference between the control and the treatment setups. The mean value for control is 2892.10 ± 0.00 mg/kg and treatment 1675.25 ± 707.79 mg/kg. The reduction in TPH showed that the test organism *P. ostreatus* has the ability to utilize hydrocarbons in the lubrication oil present in the sample as its energy source. This can be

attributed to the ability of the enzymes secreted by the fungi to breakdown complex hydrocarbons making it possible for the fungi to utilize them (Isikhuemhen *et al.*, 2003). It also indicates that mycoremediation occurred which is in line with the study of Mohammadi-Sichani *et al* (2019) and Stephen *et al* (2016) who stated that mycoremediation is linked to the breakdown of complex chemical compounds into non-hazardous and simpler forms by fungi resulting in materials of higher nutritive value. The result obtained is also in line with the work of Adenipekun and Isikhuemhen (2008) and Ibiene *et al* (2011), where a high reduction of TPH was observed during the degradation of engine oil with *Lentinus squarrosulus* Mont. (Singer).

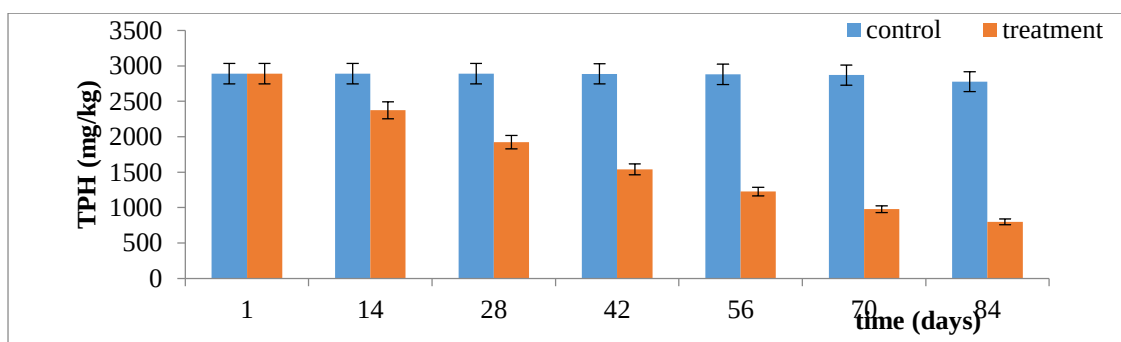


Fig 3: changes in TPH during treatment with *P. ostreatus*

The growth of *P. ostreatus* in the polluted soil increased the percentage organic carbon (%TOC) concentration in the different setups (figure 4).

There was an increase in % TOC from 3.16% (control) to 3.476 % at day 14, 3.95 % at day 28, 4.171% at day 42, 4.582% at day 56, 4.993% at day 70 and 5.4668% at day 84 resulting in a percentage gain of 10.0 %, 25.2 %, 32 %, 45.7 %, 58.1 % and 73.3 % respectively. There was significant difference between the control and treatment values. The mean value for control is 3.07 ± 0.02 % while that of the treatment is 4.26 ± 0.412 %. The increase in %TOC with increase in incubation period when compared with the controls agrees with Adenipekun *et al.* (2011); Ibiene *et al* (2011) and Adeleye *et al* (2018) who reported significant increase in %TOC during the bioremediation of battery

and cement contaminated soil by *P. pulmonaris*. The increase was attributed to the degradation of the pollutant through mycoremediation activities of the test organism which increased biomass.

The different setups were also monitored for changes in nitrate concentration and result revealed an increase from 89.48 mg/kg (control) to 95.55 mg/kg at day 14, 98.06 mg/kg at day 28, 98.83 mg/kg at day 42, 98.92 mg/kg at day 56, 98.81 mg/kg at day 70 and 84.68 mg/kg at day 84 resulting in percentage loss of 5.67 %, 9.59 %, 10.45 %, 8.25 %, 2.97 % and 5.35 % respectively. The result of this analysis is similar to the findings of Idemudia *et al* (2014) and Ogbeh *et al* (2018) who recorded an increase in nitrate concentration during the bioremediation of spent engine oil contaminated soils. This is shown in figure 5.

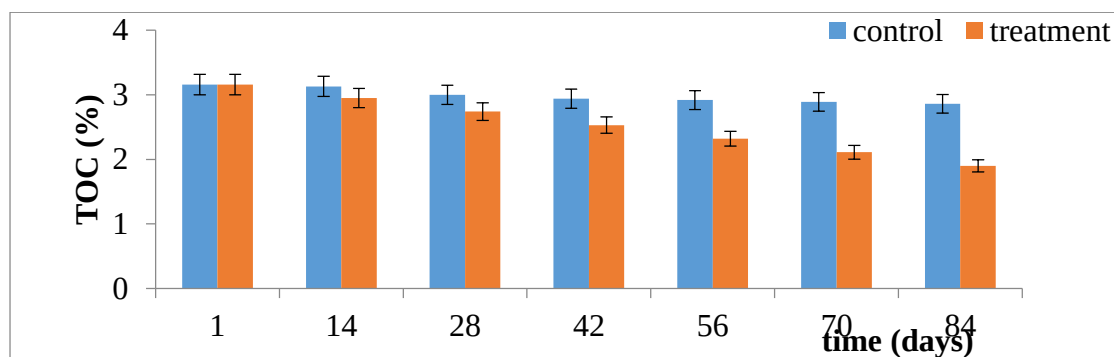


Fig 4: changes in TOC during treatment with *P. ostreatus*

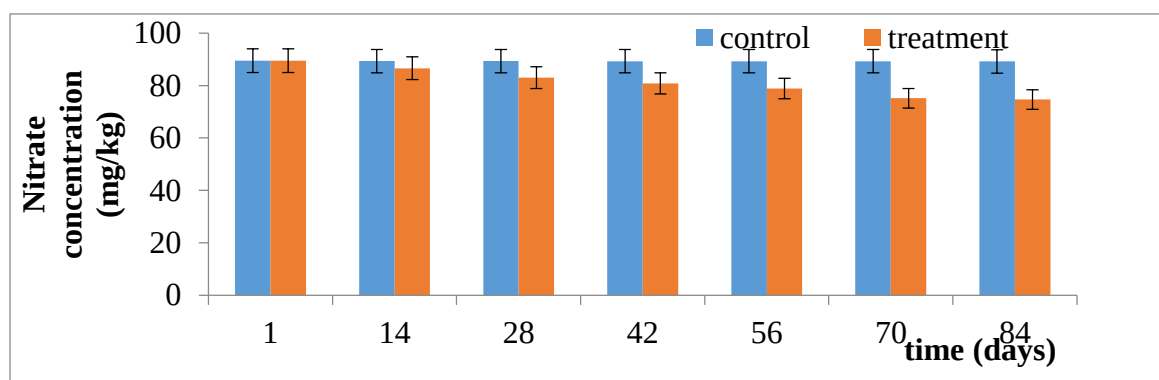


Fig 5: Changes in Nitrate concentration during treatment with *P. ostreatus*

The phosphate concentration of the control setup was 18.80 mg/kg (figure 6). The growth of *Pleurotus ostreatus* after 14 days in the treatment setups resulted in a change in the concentration of the sample to 16.73 mg/kg,

14.82mg/kg after 28 days, 12.89 mg/kg after 42 days, 10.98 mg/kg after 56 days, 9.062 mg/kg after 70 days and 7.144 mg/kg after 84 days.

The phosphate concentration at $P < 0.05$ for the two conditions were statistically different with mean value for control 18.80 ± 0.00 mg/kg and treatment 12.89 ± 3.84 mg/kg. The phosphate concentration decreased with time of treatment indicating the utilization of

phosphate by the test organism for metabolism. The result obtained conforms to the work of Zhu *et al.* (2001) and Orji *et al.* (2012) which showed that the rate of degradation is largely dependent on the carbon, nitrogen and phosphate ratio of the environment.

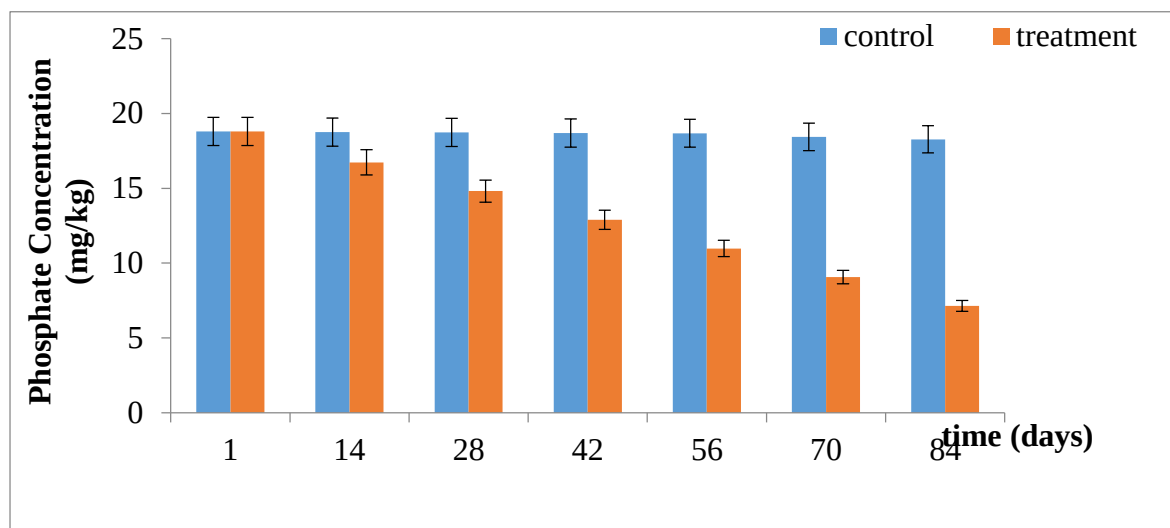


Fig 6: Changes in phosphate concentration during treatment with *P. ostreatus*

CONCLUSION

The result of this study proves that *Pleurotus ostreatus* has the potential to thrive and colonize soils contaminated with high concentrations of used lubricating oil in addition to the high adaptive nature of the isolate. *P.ostreatus* can be effectively used as a remediation agent to degrade hydrocarbon pollutants. It was shown that saw dust can be

used as a good substrate in the growth of white rot fungus for the degradation of lubricating oil contaminated soil.

Therefore, *P. Ostreatus* can be said to have proved to be an efficient, low-technology, adaptable and environmental friendly organism which can be employed in the recovery of lubricating oil and other hydrocarbon impacted soils.

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