IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 15, Issue 2 Ser. II (February 2021), PP 10-19
www.iosrjournals.org

Biostimulatory effects of *Pleurotus ostreatus* spent substrate in bioremediation of spent engine oil-contaminated soil

Nyinoh, I. W, Ejeh, I, Utume, L. N, Ada, R
[1]*Department of Biological Sciences, Faculty of Science, Benue State University, Makurdi, Benue State, Nigeria*

Abstract
Background: *Large quantities of spent crankcase oil are disposed into the environment in Nigeria, posing threats that affect plants, animals and human health. The bioremediation potential of Pleurotus ostreatus spent substrate was evaluated against spent crankcase oil-contaminated soil.*
Materials and Methods: *Fixed quantities of 100 g soil samples were mixed with variable amounts of P. ostreatus spent substrate (15, 15, 30, and 60 g) for the control and Treatments (T) 1-3. Aliquots of 0, 75, 150 and 300 ml water were introduced in the treatments to guarantee the highest possible reduction in total petroleum hydrocarbon (TPH). Microbiological assessment of soil samples and physicochemical assessment of the spent mushroom substrate were conducted.*
Results: *The indigenous hydrocarbon-utilizing bacteria (Bacillus sp., Pseudomonas sp., and Staphylococcus sp.,) and hydrocarbon-utilizing fungi (Penicillium sp., Aspergillus sp., and Rhizopus sp.) were identified from the spent crankcase oil-contaminated soil using standard microbiological procedures. The results obtained showed increases in soil pH, temperature (*℃*), moisture content (%), total organic carbon (%), total organic matter (%), and phosphorus (%), while nitrogen (%) showed a decreasing pattern across all the soil treatments. Initial Total Petroleum Hydrocarbon (TPH) of the spent engine oil-contaminated soil sample was determined to be 3784 but declined to 3064, 2286.33, 2091.67, 1227.33, and 382 mg/kg for the Control, T1, T2, T3 and T4 respectively. T4 showed better bioremediation effect due to the drastic reduction of the oil when compared with the control.*
Conclusion: *This study validates the use of Pleurotus ostreatus spent substrate as an agent for improving soil health, and as a mineral nutrient source for hydrocarbon utilizing microorganisms.*

Keywords: *Pleurotus ostreatus, spent crankcase oil, bioremediation, hydrocarbon-utilizing fungi, hydrocarbon-utilizing bacteria, Nigeria.*

Date of Submission: 06-02-2021 Date of Acceptance: 20-02-2021

I. Introduction

The release of used crankcase oil from automobiles into the environment seriously affects the natural ecosystems. Ideally, used oils are regarded as hazardous wastes because they are poisonous to the health of humans and/or the natural environment (Duru *et al.*, 2019; Ali *et al*., 2017).

Approximately one gallon of spent crankcase oil has been reported to pollute a million gallons of water including fauna and flora (Filho *et al*., 2017). Similarly, Chin *et al.*, 2012 has reported that one liter of spent automobile oil can contaminate up to 3,784 m^2 of soil, rendering it unproductive for agricultural purposes for nearly a century. Therefore, spent auto oils are either recycled and reused or disposed of in a controlled manner by adhering to legislation and policies (Dike *et al.*, 2013). Sadly, effective legislations on the management and disposal of spent oils are lacking in Nigeria. This is bolstered by assertions that in 'throw away countries', recycling of most chemicals is inadequate or absent (Collins *et al*., 2020). In Makurdi town, like elsewhere in Nigeria, they are no facilities for recycling chemicals, hence, used crankcase oil, composed of heavy metals and aromatic hydrocarbons are recklessly dumped as harmful wastes at the automobile sites without any consideration on the impact to the environment. The improper disposal of such oil carries multiple risks. For instance, with each rainfall, oil dumped on land is washed and may seep into surface and groundwater leading to water and soil pollution. Correspondingly, they may also result in adverse health effects in humans through the food chain (Ojuederie and Babalola, 2017).

Bioremediation, an eco-friendly, inexpensive and cost-effective approach to extracting toxic pollutants from the soil and wastewater, could be employed to overcome this problem. Bioremediation utilizes living organisms to remove toxic pollutants from the environment thereby restoring the ecosystem (Ayangbenro and Babalola, 2017). The key players utilized in bioremediation include earthworms (Bhat *et al.*, 2018; Ekperusi and

Aigbodion, 2015), plants (Angelova *et al*., 2016), bacteria (Ameen *et al*., 2020; Coelho *et al*., 2015; Kalaimurugan *et al*., 2020), and algae (Angulo *et al*., 2018; Leong and Chang, 2020).

The use of fungi, in a process called mycoremediation has also been documented (Goltapeh *et al*., 2013). Mycoremediation utilizes either macrofungi (mushrooms) (Ali *et al*., 2017; Chatterjee *et al*., 2017) or molds (filamentous fungi) (Deshmukh *et al*., 2016). Members of the white-rot fungi genus *Pleurotus* possess wood-decaying characteristics that could result in the decomposition of portions of the cell wall of the host plant, like, cellulose, lignin, and hemicellulose (Sanchez, 2009). *Pleurotus ostreatus* is commonly associated with dead or decaying trees e.g. beech and oaks (Bari *et al*., 2015; Karim *et al*., 2016). Besides being edible and medicinal (Tolgol, 2001), it is useful in several industrial applications. Studies by Purnomo *et al*., 2008 reported that *P. ostreatus* were employed in the degradation of the dichlorodiphenyltrichloroethane (DDT)-contaminated soil. Similarly, in other studies, *P. ostreatus* was reported to; sequester heavy metals from soils and metal scrap sites (Boamponsem *et al*., 2013; Raj *et al*., 2011; Vaseem *et al*., 2017); remove drugs from livestock waste (Migilore *et al*., 2012); in wastewater treatment to degrade contaminants like acetaminophen and sulphonamides (Chang *et al*., 2018), and petroleum-contaminated soil (Dickson *et al*., 2020; Robichaud *et al*., 2019).

According to the National Bureau of Statistics, 2018, the estimated vehicle population in Nigeria was 11.8 million. It can only be imagined the volume of used crankcase oil thrown away in the various automobile workshops in the country. However, previous data has it that yearly, above 76 million liters of spent crankcase oil is produced in Nigeria by automobile and generator workshops, besides that spilled unintentionally from engine exhaust systems during their operation (Faboya, 1997; Osubor and Anoliefo, 2003). The aim of this study is, therefore, to evaluate the role of *P. ostreatus* spent substrate in the bioremediation of spent mineral-based crankcase contaminated soil under different conditions *in vitro*. The objectives of the study are to; (i) evaluate the microbial and physicochemical properties of the crankcase oil-contaminated soil sample at Apir Mechanic Village and (ii) investigate the success of bioremediation by determining some soil parameters after bioremediation.

II. Materials and Methods

Study Area

This study was conducted in Makurdi, Benue State, located in the north-central region of Nigeria. The town is situated along the River Benue Bank on latitude 7.44˚N and longitude 8.32˚E.

Study Site

Spent crankcase oil-contaminated soil samples for this work was collected from the Apir Mechanic Village, Makurdi one of the biggest mechanic sites situated along the Makurdi-Otukpo Road. Several mechanic vendors specializing in the repair of different automobile brands characterize the automobile village.

Sample Collection

The representative soil samples were collected from four selected polluted points within the site. The Global following Positioning System (GPS) coordinates were used; Point A (7˚ 40' 36.732" N; 8˚ 32' 16.224" E), Point B (7˚ 40' 36.732" N; 8˚ 32' 16.476" E), Point C (7˚ 40' 34.14" N; 8˚ 32' 11.652" E), and Point D (7˚ 40' 34.14" N; 8˚ 32' 11.472" E). Soil samples were scooped at a tillage depth of approximately 10 cm for each point and immediately transported, to the laboratory for microbiological analysis within 24 hours of collection. The colour of the soil samples was noted, and *P. ostreatus* spent substrate was obtained from the mushroom unit of Oracle Farms Ltd. Makurdi, Nigeria.

Characterization of Oil-Contaminated Soil and *P. ostreatus* Spent Substrate

The physical and chemical evaluation of the test soil samples and spent mushroom substrate were performed using standard procedures. As described by Ogbeh *et al.,* (2019), following, collection, 100 g each of the soil samples were homogenized manually, filtered through a 2.0 mm sieve and stored for 24 h in a jute bag. The pH of soil samples, *P. ostreatus* spent substrate and all treatments were determined using a HANNA Combo pH Meter in a 1:1 mixture of sample and distilled water as described in AOAC (1990). Moisture content was determined as described in Gbarakoro and Chukwumati, (2019), using:

$$\text{Soil moisture content (\%)} = \frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{wet weight (g)}} \times 100$$

Percentage nitrogen, organic carbon, and phosphorus were determined using methods previously described by Association of Analytical Chemists (2009). Percentage organic matter was determined by multiplying organic carbon (%) with 1.724 as previously described (Gbarakoro and Chukwumati, 2019). Temperature (°C) was measured using a digital thermometer as described (Gbarakoro and Chukwumati, 2019).

Estimation of Total Petroleum Hydrocarbon (TPH)

Total petroleum hydrocarbon (TPH) was determined via oil and grease content measurement using the procedure by Abdulyekeen et al., (2016). Residual oil/grease content was finally determined using the equation a and b:

Gain in weight of flask mg = w1 − w2 (a)

Oil and grease fraction mg kg = $\frac{w1 - w2}{W} \times 100$ (b)

W1 = weight of flask, boiling chips and residue after evaporation of hexane W2 = weight of round flask and boiling chips W = the weight of the contaminated soil in gramme

Percentage degradation/remediation of total petroleum hydrocarbon (%) was determined using:

Initial TPH-Residual TPH Initial TPH × 100

Enumeration of Aerobic Heterotrophic Bacteria and Fungi

One gram (1.00 g) of soil sample was suspended in 9.00 ml of sterile distilled water and ten-fold serial dilution of the resulting homogenized mixture was carried out. Aliquots of 1.00 ml were plated unto sterile Petri dishes containing nutrient agar (HiMedia, Mumbai, India) supplemented with nystatin to suppress fungal growth. The nutrient agar plates were incubated at 30 ℃ for 24 - 48 hours. For fungal growth, appropriate dilutions were plated on potato dextrose agar (PDA) (HiMedia, Mumbai, India) agar supplemented with streptomycin to suppress bacterial growth. Incubation was for up to a week at 30 ℃. The numbers of viable microorganisms were mathematically determined using the expression below:

CFU/g = $\frac{\text{average no. of colonies x total dilution factor}}{\text{volume plated}}$

Enumeration of Hydrocarbon Utilizing Bacteria and Fungi

Mineral salt agar (LSBio, San Diego, USA) was used (Mills *et al.*, 1978; Okpokwasili and Amanchukwu, 1988). A 10 ml volume of crude oil was amended to 990 ml of mineral salt medium in conical flasks to serve as carbon source. Incubation was carried out for bacteria at 30 ℃ for 24 - 48 hours Aliquots were plated on medium supplemented with nystatin to suppress fungal growth. For Hydrocarbon Utilizing Fungi (HUF), aliquots were plated unto medium supplemented with streptomycin and gentamycin to suppress bacterial growth and incubated for up to a week. Numbers of cells/g were enumerated.

Morphological and Biochemical Identification of Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell micromorphology. Catalase, Citrate, Indole, Urease, Oxidase, and Triple Sugar Iron (TSI) agar reaction via Hydrogen Sulphide (H_2S) production were also employed in the characterization and identification of bacterial isolates as described by Adeoye (2007); Cheesbrough (2005); Holt *et al.* (1994). Microbial identification was carried out using guidelines and keys from Holt *et al*., (1994). Microscopic and macroscopic examination via the needle mount technique was performed on fungal isolates and their identification was carried out as described (Barnett *et al.*, 1972; Larone, 1972).

Setup of Bioremediation experiments

The bioremediation experiment was performed in the Microbiology Laboratory, Benue State University Makurdi, Nigeria from June – July 2019. This experiment was carried out using four (4) treatments, and three (3) replicates each and was maintained at a constant temperature of 30 ℃ throughout the bioremediation period. The following treatment groups (Table 1) were investigated.

Table 1: Experimental Setup

Treatment Number	Number of Replicates	Contaminated Soil (g)	*Pleurotus ostreatus* Spent Substrate (g)	Water (ml)
01	Three (3)	100.00	15.00	0.00
02	Three (3)	100.00	15.00	75.00
03	Three (3)	100.00	30.00	150.00
04	Three (3)	100.00	60.00	300.00

III. Results and Discussion

Evaluation of Soil and *P. ostrearus* Spent Substrate Quality

Table 2 shows the results for the key parameters of the oil-contaminated soil and *P. ostreatus* spent substrate.

Table 2: Characteristics of Oil-contaminated soil and *P. ostreatus* spent substrate before bioremediation

Measured Parameters	**Cranckcase oil-contaminated soil**	***P. ostreatus* spent substrate**
Hydrogen Ion Concentration (pH)	6.40	7.30
Moisture Content (% dry weight)	4.69	6.60
TOC (% dry weight)	0.97	35.66
TOM (% dry weight)	1.67	61.48
Nitrogen (% dry weight)	0.39	0.96
Phosphorus (% dry weight)	1.33	1.86
Temperature (◦C)	24	27.00
Aerobic Heterotrophic Bacteria (cfu/g)	2.01×10^7	1.88×10^7
Aerobic Heterotrophic Fungi (cfu/g)	8.00×10^5	-
Aerobic HUB (cfu/g)	1.4×10^6	-
Aerobic HUF (cfu/g)	3.00×10^5	-
TPH (mg/kg)	3,784	Below Detectable Limit (BDL)
Colour	Blackish or dark-brownish	Whitish-Brown

TOC: Total Organic Carbon; TOM: Total Organic Matter; HUB: Hydrocarbon Utilizing Bacteria; HUF: Hydrocarbon Utilizing Fungi; Total Petroleum Hydrocarbon; - : not determined.

Soils in Makurdi automobile workshops are largely polluted, due in part to the improper disposal of spent crankcase oils in the environment. As suggested by Ramadas *et al*., (2015) spent oils top the most hazardous classes of pollutants contaminating the environment. These products permeate the soil resulting to changes in the physical and chemical qualities of the soil (Griffiths and Philippot, 2013), and thus deterioration of these soil characteristics (Semrany *et al*., 2012), as we have observed in our study (Table 2).

The soil samples characterization revealed its state before bioremediation. At a pH of 6.4, the soil samples were only slightly acidic; while *P. ostreatus* spent substrate had a neutral pH of 7.3. Studies by Ogbeh *et al*., 2019 on the bioremediation of spent engine-oil contaminated soil in Makurdi also measured a similar pH of 6.25. Next, the moisture content of the soil on a dry weight basis was determined. Low moisture contents in both the oil-contaminated soil samples (4.69%) and the *P. ostreatus* spent substrate (6.60%). The total organic carbon (TOC), a measure of microbial biomass; total organic matter (TOM), the organic component of soil, the nitrogen, and phosphorus levels, which are vital nutrients in soil fertility were all evaluated.

There were pronounce contrasts between the soil samples versus the *P. ostreatus* substrate in TOC, TOM, nitrogen, and phosphorus quantities. Soils lacking in nutrients have been found to have low organic matter. As such, the low SOM, nitrogen and phosphorus in the soil samples were expected. The numbers of culturable aerobic heterotrophic bacteria in soil is proportional to the activity of microbes, and also indicates soil health. Although oil-contaminated soils are unproductive for agricultural purposes since plants grow poorly on them (Wyszkowska *et al*., 2015), still, they may serve as sources of energy for certain microbes, especially the hydrocarbon-utilizers. This may explain why in this study, although other soil physical and chemical parameters investigated in this study revealed low values, aerobic heterotrophic bacteria were the most abundant microbial group in the oil-contaminated soil with an average CFU count/g of 2.01×10^7 (Table 2). By colour, the soil at the mechanic village had a blackish or dark-brownish soil colouration, thus highlighting possible spent oil-contamination.

Microbiological Diversity of Spent Mineral-based Crankcase Oil-contaminated Soil

Biochemical tests were also conducted on the oil-contaminated soil to identify the indigenous bacteria (Table 3) and fungi present (Table 4) at the polluted sites.

Table 3 indicates that bacteriological analysis detected the presence of heterotrophic bacteria.

Table 3: Occurrence of bacteria from crankcase oil-contaminated soil samples

Colony Morphology	Cell Arrangement	GR	CA	OX	CI	IN	UR	TS	Organism Suspected	% Occurrence
Smooth colonies with grey tint on nutrient agar	Short rods	-	+	+	+	+	+	H_2S	*Pseudomonas* sp.	33.33%
Raised long rods	Straight long	+	+	_	+	+	-	A/G	*Bacillus* sp.	33.33%
Milky to white colonies	Cocci in clusters	+	+	-	-	-	-	A	*Staphylococcus* sp.	16.67%
Large smooth and circular slightly raised colonies	Short rods	-	+	-	+	-	+	H_2S	*Klebsiella* sp.	16.67%

Key: GR: Gram Stain; CA: Citrate Test; OX: Oxidase Test; CI: Citrate Test; IN: Indole Test; UR: Urease Test; TS: Triple Sugar Iron Reaction; H_2S: Hydrogen Sulphide; A: Acid Production; A/G: Acid and Gas Production; +: Positive; - : Negative

Pseudomonas sp., *Bacillus* sp., *Staphylococcus* sp., and *Klebsiella* sp., were isolated from the oil-contaminated soil samples, however only *Klebsiella* sp., was not screened as HUB. *Staphylococcus*, *Bacillus* and *Pseudomonas* sp., have been reported to have the ability to degrade hydrocarbon in diesel-contaminated soil environment (Nikhil *et al.*, 2013). The percentage occurrence of the detected bacteria reveals that *Bacillus* and *Pseudomonas* had the highest percentage occurrence of 33.33 %, with *Staphylococcus* and *Klebsiella* at 16.67%. Fungi were isolated from the crankcase oil-contaminated soil. As shown in Table 4, *Penicillium* sp., *Aspergillus* sp., and *Rhizopus* sp., were detected and they were all screened as HUF.

Table 4: Diversity of fungi from crankcase oil-contaminated soil samples

Colony Morphology	Microscopic Morphology	Identification	% Occurrence
Olive grey, velvety or cottony	Septate hyaline hyphae, branched conidiophores, metulae and conidia	*Penicillum* sp.	50%
Dark grey surface, reverse velverty or cottony	Non septate, conidiophores terminating in a globus or clarate swelling bearing phialides at the apex	*Aspergillus* sp.	25%
Colony pale brownish grey, reverse brownish black	Branched rhizoids sporangiospore irregular in shape, globolose and interwoven mass of hyphae	*Rhizopus* sp.	25%

For the percentage occurrence, *Penicillium* sp., had the highest occurrence at 50 %, while *Aspergillus* sp., and *Rhizopus* sp., had the least occurrence at 25 % each. Studies by Barnes *et al.*, 2018 also identified fungi of the *Aspergillus* sp., and *Penicillium* sp., genus as HUF with bioremediation potentials, so as studies by Adams *et al.*, (2014), Eziuzor and Okpokwasili (2009), Obiakalaije *et al.*, (2015) and Vidali, 2001 in their bioremediation studies.

Physicochemical Analysis of Soil during Mycoremediation

Juwarkar et al., (2010) suggested that in bioremediation, experiments are optimized, by exploiting factors such as nutrient inclusion, soil structure, moisture, and aeration. With that goal, our bioremediation setup varied the amounts of spent mushroom substrate and moisture content (Table 1) at a constant temperature of 34 °C.

At the start of the bioremediation experimentation, and throughout the process, the pH, a measure of the acidity or alkalinity was measured for the control and treatment groups (Figure 1).

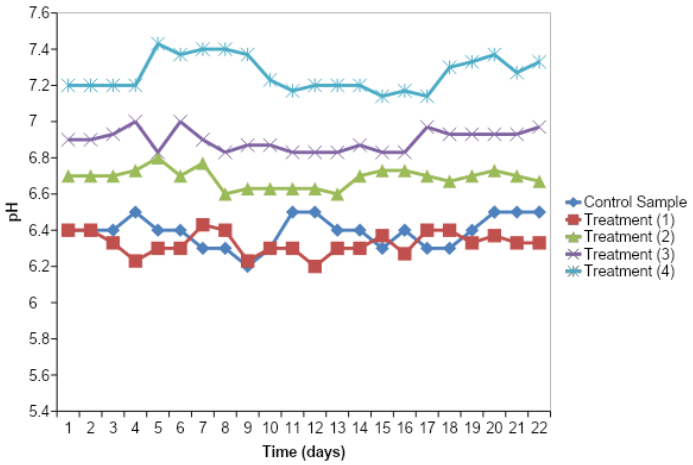

Figure 1: pH versus days of remediation of crankcase oil-contaminated soil

pH is an important factor influencing biological activities and hence the rate of bioremediation. As shown in Figure 1, the mean pH values across all treatments ranged from 6.33 – 7.26. Most microbes have been reported to perform optimally at a neutral pH range (Ajoku and Oduola, 2013). A pH of 6.5 – 7.5 has been shown for both laboratory and field studies, to be ideal for the performance of hydrocarbon-degrading bacteria (Eweis *et al*., 1998; EPA, 1993). This study agrees with these findings, as the observed mean pH values were within this range.

Bioremediation is an enzymatic process, and the temperature is a critical factor that influences its rate by controlling the rate of enzyme-catalyzed reaction within the microorganisms. The mean temperature (℃) values of the soil samples were measured across all the treatments during remediation as indicated (Figure 2).

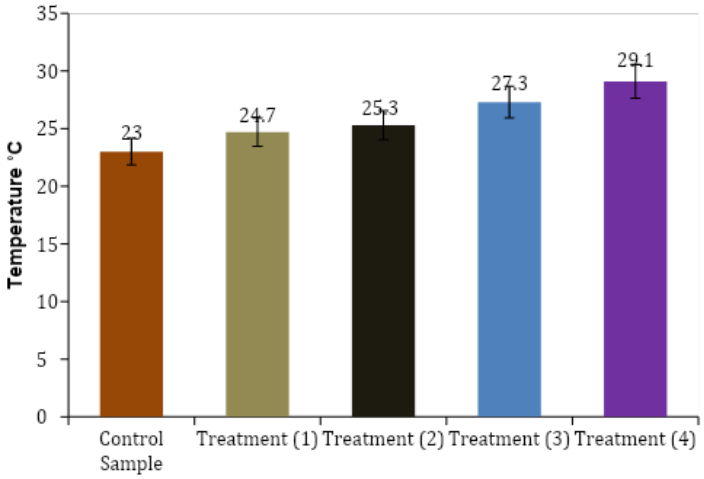

Figure 2: Temperature versus bioremediation treatments
Error bars represent mean of three measurements

The results show that a slight temperature increases across the treatments, beginning at 23 ℃ (control) to 24.7, 25.3, 27.3 to 29.1 ℃ for treatment 1 – 4 respectively were observed. Nester *et al*., 2001, reported that most soil bacteria including HUB are mesophilic and thrive best at 25 – 45 ℃. Therefore, it is not surprising that treatment 4 that showed the highest temperature of 29.1 ℃ which is within this range showed the best bioremediation result. In studies by Gbarakoro and Chukwumati (2019), temperature increase (25.5 ℃ – 27 ℃) across their treatments was also reported.

Moisture is required by all microorganisms for proliferation and activity, as such moisture content is another key factor in bioremediation. We evaluated the mean moisture content for all the treatments during remediation and the results are presented in Figure 3.

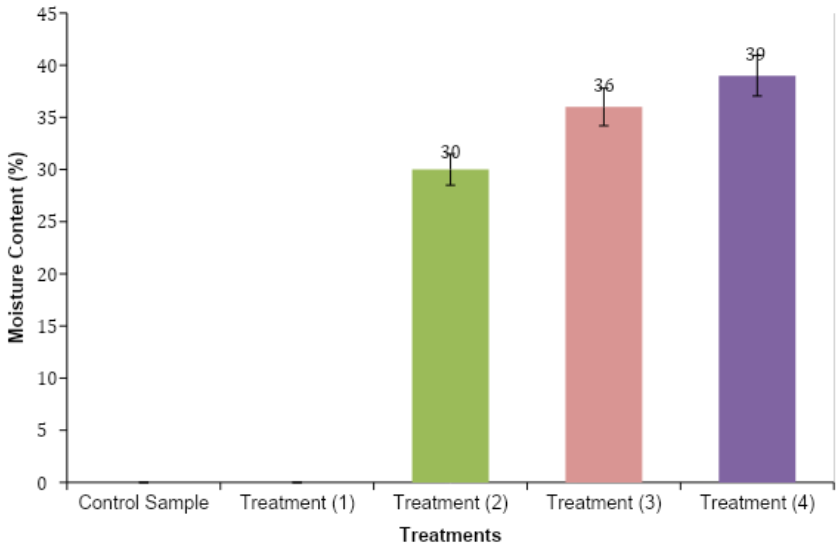

Figure 3: Mean moisture Content (%) versus bioremediation treatments

Moisture content (%) results from this study indicates a range of 30 %, 36 %, and 39 % respectively across treatments 2, 3 and 4 to which 75, 150, and 300 ml water (Table 1) were added throughout bioremediation. The variation of soil moisture content is essential in successful bioremediation on contaminated sites (Cho *et al*., 2000), and importantly, two major outcomes are likely; (i) limited quantities of water inhibits proliferation and microbial activity whereas (ii) excessive water can be restrictive in supplying oxygen in delivering oxygen to the microbes, hence leading to a decline in decomposition rate of organic matter. Consequently, the determination of optimum water content is critical for bioremediation. As for the control, no water was added. However, although only 75 ml of water was added to treatment 1, it is suspected that the limited water supply prevented the growth of the microbes, resulting in 0% moisture content. Banerjee et al., 2016 have reported that in the effective bioremediation of oil-contaminated soil, variations of moisture from 30 % – 90 % are required, yet this depends on the type of soil and pollutants amongst other factors. The findings of this study support this, as when the volume of water was doubled in the treatments to 300 ml, the result was a decrease in TPH from 3064 mg/kg at the beginning of bioremediation to 382 mg/kg.

The total petroleum hydrocarbon (TPH) was estimated via the oil and grease method and the results are expressed in mg/kg (Figure 4).

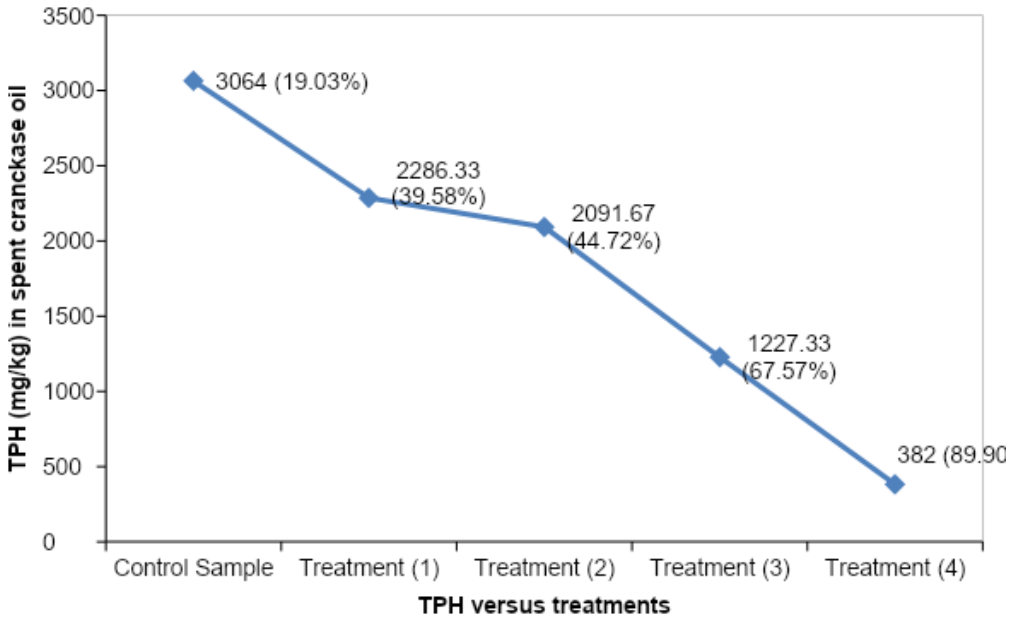

Figure 4: Total Petroleum Hydrocarbon (mg/kg) versus treatments after 22 days of bioremediation (Percentage Degradation Remediation Process)

Although the experiment lasted for only 22 days, (which may not seem to be a long time to observe changes), there was a drastic decline in the TPH, up to 89.9 % by day 22, for treatment 4. As shown in Figure 4, there was a gradual reduction to 3064 mg/kg, 2286.33 mg/kg, 2091.67 mg/kg, 1227.33 mg/kg, and 382 mg/kg for Control category, Treatment 1, Treatment 2, Treatment 3 and Treatment 4 respectively. The difference in percentage degradation (%) across Treatment 1 and Treatment 2 highlights the role played by water in this study. Treatment 1 had an equal composition of soil sample and *P. ostreatus* spent substrate, except for water, which was only added to Treatment 2, yielding a percentage degradation of 44.72 % in Treatment 2 against 39.58 % in Treatment 1. This, therefore, establishes the relevance of moisture in bioremediation processes. The highest percentage degradation (%) value for this study stands at 89.90 % for Treatment 4 and this is relatively in agreement with Stanley *et al*., (2017) and Adenipekun and Ogunjobi, (2011) who also reported a very high percentage loss of about 90 % of Total Petroleum Hydrocarbon in their studies.

This study investigated the improvement of key soil parameters following bioremediation by measuring TOM, TOC, Nitrogen, and Phosphorus (Figure 5).

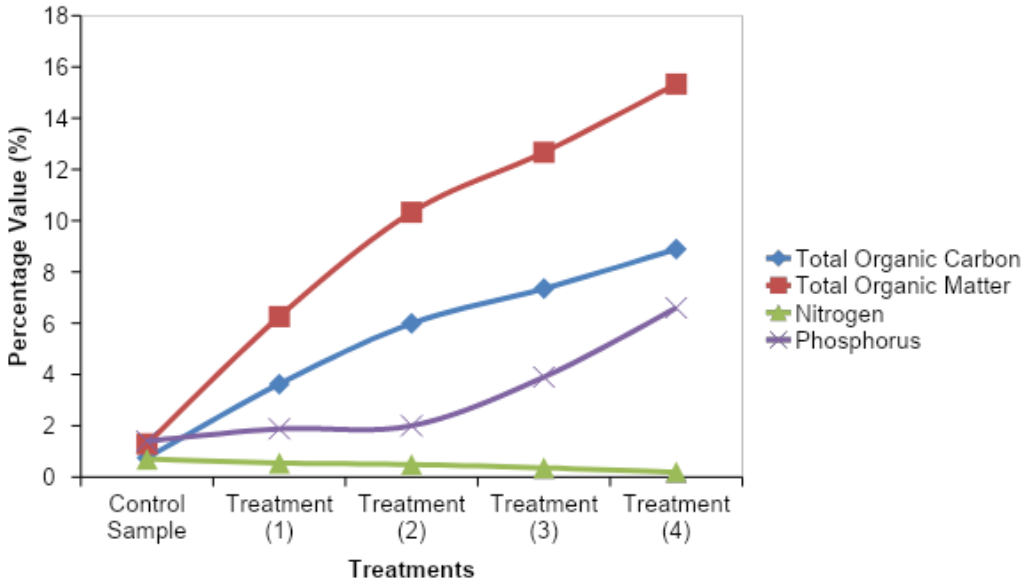

Figure 5: Key soil parameters for the control and treatment groups following bioremediation

Results showed TOC (%) steadily increased across the control category and treatments following bioremediation, and this was observed to be directly related to the increasing amounts of *P. ostreatus* spent substrate (g) and water (ml). The least TOC was 0.75, observed in the control soil sample, to which no amendments were made, and the highest TOC was 8.89, shown in Treatment 4, which had the highest amount of spent mushroom substrate and water amendments. The rise in TOC from this study is in agreement with studies by Ibiene *et al.*, (2011) and Adenipekun and Ogunjobi (2011), who reported increases in TOC in their bioremediation studies. The Nitrogen levels declined across all the treatment groups, except in the control where the highest Nitrogen value of 0.70 was recorded. This value was higher than the initial Nitrogen value of the contaminated soil sample 0.39 (Table 2). The pattern of Nitrogen decrease in this study (Figure 5) correlates with Obiakalaije *et al.*, (2015) who also reported a decreasing pattern in Nitrogen levels across treatment categories. Studies by Adams *et al.*, 2014, and Gbarakoro and Chukwumati, (2019) reported relative increases in Nitrogen across treatment categories.

Phosphorus levels (%) steadily increased across the treatment groups with a corresponding relationship to increasing amounts of *P. ostreatus* spent substrate (g) and water (ml). Hence, the least Phosphorus value; 1.40, was obtained in the control category, to which no amendment was made. The highest value for Phosphorus; 6.60, was obtained in Treatment 4 which had the highest amount of amendments (*P. ostreatus* spent substrate (g) and water (ml) across the treatments. The increasing pattern of Phosphorus from this study correlates with Gbarakoro and Chukwumati, (2019) who reported an increase in Phosphorus across treatment categories. Obiakalaije *et al.* (2015) however reported a pattern of decrease across treatment categories.

IV. Conclusion

Soil contaminated with crankcase oil-contaminated soil is characterized by low physicochemical parameters such as moisture content, TOC, TOM, nitrogen, and phosphorus levels, yet a higher count of bacteria and fungi. Neutral pH of 7.2, the temperature of 29.1 ℃, moisture content of 39 % yielded the best bioremediation results, with decreases in TPH from 3064 mg/kg at the beginning of bioremediation to 382 mg/kg by the end of experimentation. Except for Nitrogen, there were improvements in soil health for instance TOC, TOM, temperature, pH, and phosphorus following bioremediation. Overall, the result of this study shows *P. ostreatus* spent substrate is an ideal biostimulant in the remediation of spent mineral-based crankcase oil-contaminated soil.

Funding

Financial support for the conduct of this research was received from Petroleum Technology Development Fund (PTDF) Nigeria, being student sponsorship. The sponsors had no role in the study design, collection, analysis and interpretation of data, writing the report and in the decision to submit for publication.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this work.

Acknowledgment

We thank all the laboratory staff at the Department of Biological Sciences, Microbiology Unit, Benue State University, Makurdi, Nigeria for their technical assistance.

References

[1]. Abdulyekeen, K. A., Muhammad, I. M, S. O Giwa, Abdulsalam, S., 2016. Bioremediation of Used Motor Oil Contaminated Soil Using Elephant and Horse Dung as Stimulants. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). ISSN: 2319-2399.Volume 10, Issue 12 Ver. II, pp 73-78.
[2]. Adams, G. O, Tawari-Fufeyin, P. Ehinomen, I., 2014. Bioremediation of Spent Oil Contaminated Soils Using Poultry Litter. Research Journal in Engineering and Applied Sciences 3(2): 124-130.
[3]. Adenipekun, C. O, Ogunjobi, A. A., 2011. Bioremediation of cutting fluids contaminated soil by pleurotus tuberregion. The Environmentalist; 32:11-18.
[4]. Adeoye, A., 2007. Medical Laboratory Practice, 1st edition FEMCO Publishers Limited, Lagos, Nigeria, pp: 149-153.
[5]. Ajoku, G. A. O., Oduola, M. K., 2013. Kinetic model of pH effect on bioremediation of crude petroleum contaminated soil. 1. Model development. Am J Chem Eng. 1(1): 6-10.
[6]. Ali, A., Guo, O. D., Mahar, A., Wang, P., Shen, F., Li, R et al., 2017. Mycoremediation of potentially toxic trace elements—A biological tool for soil cleanup: A review. Pedosphere 27(2):205-222. doi: 10.1016/S1002-0160(17)60311-4.
[7]. Ameen, F. A, Hamdan, A. M., El-Naggar, M. Y., 2020. Assessment of the heavy metal bioremediation efficiency of the novel marine lactic acid bacterium, Lactobacillus plantarum MF042018. Scientific Reports 10 (314).
[8]. Angelova, V. R., Perifanova-Nemska, M., Uzunova, G., Ivanov, K., Lee, H., 2016. Potential of sunflower (Helianthus annuus L.) for phytoremediation of soils contaminated with heavy metals. World J. Sci. Eng. Technol. 10:1–11.
[9]. Angulo, E., Bula, L., Mercado, I., Montano, A., Cubillan, N., 2018. Bioremediation of Cephalexin with non-living Chlorella sp., biomass after lipid extraction. Bioresour Technol. 257:17-22. doi: 10.1016/j.biortech.2018.02.079.
[10]. Association of Analytical Chemists (AOAC)., 2009. Official Methods of Analysis of the Association of Official Analytical Chemists. Washington, DC.
[11]. Ayangbenro, A. S., Babalola, O. O., 2017. New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. Int J Environ. Res. Public Health; 19; 14(1).
[12]. Banerjee, A., Roy, A., Dutta, S., Mondal, S., 2016. Bioremediation of hydrocarbon—A review. *Int. J. Adv. Res. 4*, 1303–1313.
[13]. Bari, E., Nazarnezhada, N., Kazemia, S. M., Ghanbaryc, M. A. T., Mohebbyd, B., Schmidte, O., Clausenf, C. A., 2015. Comparison between degradation capabilities of the white rot fungi Pleurotus ostreatus and Trametes versicolor in beech wood. International Biodeterioration and Biodegradation 104:231–237. doi: 10.1016/j.ibiod.2015.03.033.
[14]. Barnes, N. M., Khodse, V. B., Lotlikar, N. P., Meena, R. M., Damare, S. R., 2018. Bioremediation potential of hydrocarbon-utilizing fungi from select marine niches of India. 3 Biotech 8(1): 21. doi: 10.1007/s13205-017-1043-8.
[15]. Barnett, H. L., Hunter, B. B., 1972. Illustrated genera of imperfecti fungi, 3rd edition Burgess Publishing Company, Minnesota, U.S.A, pp: 119-121.
[16]. Bhat, S. A., Singh, S., Singh, J., Kumar, S., Bhawana, Pal Vig, A., 2018. Bioremediation and detoxification of industrial wastes by earthworms: Vermicompost as powerful crop nutrient in sustainable agriculture. Bioresour Technol. 252:172-179. doi: 10.1016/j.biortech.2018.01.003.
[17]. Boamponsem, G. A., Obeng, A. K., Osei-Kwateng, M., Badu, A. O., 2013. Accumulation of heavy metals by Pleurotus ostreatus from soils of metal scrap sites. Int J Curr Res Rev. 5(4):01–09.
[18]. Chang, B. V., Fan, S. N., Tsai, Y. C., Chung, Y. L., Tu, P. X., Yang, C. W., 2018. Removal of emerging contaminants using spent mushroom compost. Sci Total Environ 1; 634:922-933. doi: 10.1016/j.scitotenv.2018.03.366.
[19]. Chatterjee, S., Sarma, M. K., Deb, U., Steinhauser, G., Walther, C., Gupta, D. K., 2017. Mushrooms: From nutrition to mycoremediation. Environmental Science and Pollution Research; 24:19480-19493. doi: 10.1007/s11356-017-9826-3.
[20]. Cheesbrough, M., 2005. District Laboratory Practice in Tropical Countries. Cambridge University Press, United Kingdom, pp. 25-41.
[21]. Chin, S., Shafiq, N., Nuruddin, M., 2012. Effects of used engine oil in reinforced concrete beams: the structural behaviour. World Academy of Science, Engineering and Technology 63:932–938.
[22]. Cho, Y., Rhee, S-K., Lee, S-T., 2000. Effect of soil moisture on bioremediation of chlorophenol-contaminated soil. Biotechnol Lett. 22:915–9.
[23]. Coelho, L. M., Rezende, H. C., Coelho, L. M., de Sousa, P. A., Melo, D. F., Coelho, N. M., 2015. Bioremediation of polluted waters using microorganisms. In: Shiomi N., editor. Advances in Bioremediation of Wastewater and Polluted Soil. InTech; Shanghai, China.
[24]. Collins, C., Depledge, M., Fraser, R., Johnson, A., Hutchinson, G., Matthiessen, P., Murphy, R., Owen, S., Sumpter, J., 2020. Key actions for a sustainable chemicals policy. Environ. Int. 137:105463. doi: 10.1016/j.envint.2020.105463.
[25]. Deshmukh, R., Khardenavis, A. A., Purohit, H. J., 2016. Diverse metabolic capacities of fungi for bioremediation. Indian Journal of Microbiology 56(3):247-264. DOI: 10.1007/s12088-016-0584-6.
[26]. Dickson, U. J., Coffey, M., Mortimer, R. J., Smith, B., Ray, N., Di Bonito, M., 2020. Investigating the potential of sunflower species, fermented palm wine and Pleurotus ostreatus for treatment of petroleum-contaminated soil. Chemosphere 240:124881. doi: 10.1016/j.chemosphere.2019.124881.
[27]. Dike, B. U., Okoro, B. C., Nwakwasi, N. N., Agbo, K. C., 2013. Remediation of Used Motor Engine Oil Contaminated Soil: A Soil Washing. J. Civil Environ. Eng. 3:1.
[28]. Duru, C. E (2019) Assessment and modeling of heavy metal pollution of soil within reclaimed auto repair workshops in Orji, Imo State Nigeria. Chem J Moldova 14(1):54–60.
[29]. Ekperusi, O. A., Aigbodion, F. I., 2015. Bioremediation of petroleum hydrocarbons from crude oil-contaminated soil with the earthworm: Hyperiodrilus africanus. 3 Biotech. 5(6):957-965.
[30]. EPA, Guide for conducting Treatability studies under CERCLA. Biodegradation Remedy Selection. EPA/540/R-03/519 a, 1993, pp 1-41.
[31]. Eweis, B. J., Savina, J. E., Daniel, P. Y. C., 1998. Edward DS. Bioremediation principles. International Edition, McGraw-Hill Company, Inc. United States.

[32]. Eziuzor, C. S., Okpokwasili, G. C., 2009. Bioremediation of hydrocarbon contaminated mangrove soil in a bioreactor. Nig. J. Microbiol. 23 (1): 1777-1791.
[33]. Faboya, O.O. P., 1997. Industrial pollution and waste management. Akinjide O, editor. Dimensions of environmental problems in Nigeria. Washington D.C: Friedrich Ebert Foundation; pp. 12–25.
[34]. Filho, J. L.A., Moura, L. G. M., Ramos, A. C. S., 2010. Liquid extraction and adsorption on solid surfaces applied to use lubricant oils recovery. Brazilian J Chem Eng. 27:687–697.
[35]. Gbarakoro, T. N., Chukwumati, J., 2019. Short-Term Remediative Impact of Spent Mushroom Substrate on Soil Nutrients in Spent Automobile Lubricant Habitat-Types at University Farm, University of Port Harcourt, Nigeria. International Journal of Ecosystem 9(1): 1-5.
[36]. Goltapeh, E. M., Danesh, Y. R., Varma, A., editors. Fungi as Bioremediators. 1st ed. Berlin/Heidelberg: Springer-Verlag; 2013.
[37]. Griffiths, B. S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37: 112–129. doi: 10.1111/j.1574-6976.2012.00343.
[38]. Holt, J. G., Kreig, N. R., Sneath, P. H. A., Stanley, J. T., William, S. T., 1994. Bergey's manual of Determinative Bacteriology. Baltimore, USA: William and Wikins, pp. 722-783.
[39]. Juwarkar, A. A., Singh, S. K., Mudhoo, A. A., 2010. Comprehensive overview of elements in bioremediation. Reviews in environmental science and Bio/Technology 9; pp 215–288.
[40]. Kalaimurugan D., Balamuralikrishnan, B., Durairaj, K., Vasudhevan, P., Shivakumar, M. S., Kaul, T., Chang, S. W., Ravindran, B., Venkatesan, S., 2020. Isolation and characterization of heavy-metal-resistant bacteria and their applications in environmental bioremediation. International Journal of Environmental Science and Technology, 17, pp 1455–1462.
[41]. Karim, M., Daryaei, M. G., Torkaman, J., Oladi, R., Ghanbary, M. A. T, Bari, E., 2016. In vivo investigation of chemical alteration in oak wood decayed by Pleurotus ostreatus. International Biodeterioration and Biodegradation, 108:127–132. doi: 10.1016/j.ibiod.2015.12.012.
[42]. Larone, B. H., 1972. Important Fungi: A Guide to Identification, Harper and Row Publishers, Hagerstown, Maryland, pp 5-26.
[43]. Leong, Y. K., Chang, J. S., 2020. Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. Bioresour Technol. 303:122886. doi: 10.1016/j.biortech.2020.122886.
[44]. Migliore, L., Fjori, M., Spadoni, A., Galli, E., 2012. Biodegradation of oxytetracycline by Pleurotus ostreatus mycelium: a mycoremediation technique. J Hazard Mater, 15;215-216:227-32. doi: 10.1016/j.jhazmat.2012.02.056.
[45]. Mills, A., Breuil, L. C., Colwell, R. R., 1978. Enumeration of petroleum degrading marine and estuarine microorganisms by the most probable number method. Can. J. Microbiol. 24: 550-557.
[46]. National Bureau of Statistics. Road Transport Data Q2 2018. Available online at: http://www.nigerianstat.gov.ng/pdfuploads/Road_Transport_Data_-_Q2_2018.pdf (accessed June 2020).
[47]. Nester, Eugene W., Denise G. Anderson, C. Evans Roberts Jr., Nancy N. Pearsall, and Martha T. Nester. 2001. Microbiology: A Human Perspective. 3 rd ed. New York: McGraw-Hill.
[48]. Nikhil, T., Deepa, V., Rohan, G., Satish, B., 2013. Isolation, Characterization and Identification of Diesel Engine Oil Degrading Bacteria from Garage Soil and Comparison of their Bioremediation Potential. International Research Journal of Environment Sciences, 2(2): 48-52.
[49]. Obiakalaije, U. M., Makinde, O. A., Amakoromo, E. R., 2015. Bioremediation of Crude Oil Polluted Soil Using Animal Waste. International Journal of Environmental Bioremediation & Biodegradation, 3(3): 79-85.
[50]. Ogbeh, G. O., Tsokar, T. O., Salifu, E., 2019. Optimization of nutrients requirements for bioremediation of spent-engine oil contaminated soils. Environ. Eng. Res. 24(3).
[51]. Ojuederie, O. B., Babalola, O. O., 2017. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. Int J Environ Res Public Health 4;14(12). doi: 10.3390/ijerph14121504.
[52]. Okpokwasil, G. C., Amanchukwu, S. C., 1988. Petroleum Hydrocarbon degradation by Candida Species. Environ. Intl., 14: 239-247.
[53]. Osubor, C. C., Anoliefo, G. O., 2003. Inhibitory effect of spent lubrication oil on the growth and respiratory function of Arachis hypogea L. Benin Sci Dig. 1:73–79.
[54]. Purnomo, A. S., Kamei, I., Kondo, R., 2008. Degradation of 1,1,1- trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) by brown-rot fungi. J. Biosci. Bioeng. 105: 614-621.
[55]. Raj, D. D., Mohan, B., Vidya Shetty, B. M., 2011. Mushrooms in the remediation of heavy metals from soil. Int J Environ Pollut Control Manag. 3(1): 89–101.
[56]. Ramadass, K., Megharaj, M., Venkateswarlu, K., 2015. Ecological implication of motor oil pollution: earthworm survival and soil health. Soil Biology and Biochemistry, 85: 72–81.
[57]. Robichaud, K., Girard, C., Dagher, D., Stewart, K., Labrecque, M., Hijiri, M., Amyot, M., 2019. Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North. Chemosphere, 220:47-55. doi: 10.1016/j.chemosphere.2018.12.108.
[58]. Sanchez, C., 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnology Advances, 27:185–194. doi: 10.1016/j.biotechadv.2008.11.001.
[59]. Semrany, S., Faviera L., Djelal, H., Tahac, S., Amranea, A., 2012. Bioaugmentation: possible solution in the treatment of bio-refractory organic compounds (Bio-ROCs). Biochem. Eng. J, 69: 75–78. doi: 10.1016/j.bej.2012.08.017.
[60]. Stanley, H. O, Offorbuike, O. M., Stanley, C. N., 2017. Bioremediation of crude oil contaminated soil using Pleurotus pulmonarius, a whiterot fungus. IOSR Journal of Environment Science, Toxicology and Food Technology 11(4):122-128.
[61]. Tolgol L,Y., 2001. The species resource and ecological distribution of Pleurotus in China. Edible Fungi of China 20:8–10.
[62]. Vaseem H, Singh, V. K., Singh, M. P., 2017. Heavy metal pollution due to coal washery effluent and its decontamination using a macrofungus, Pleurotus ostreatus. Ecotoxicol. Environ. Saf. 145:42-49. doi: 10.1016/j.ecoenv.2017.07.001.
[63]. Vidali, M., 2001. Bioremediation: An overview. Journal of Applied Chemistry 73(7): 1163- 1172.
[64]. Wyszkowska, J., Kucharski, J., 2005. Correlation between the number of cultivable microorganisms and soil contamination with diesel oil. Pol. J. Environ. Stud. 14:347–356.