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Potential Use of Mushrooms and Spent Substrate in Decontamination and Cleanup of Oil Contaminated Soil: A Mini Field Climate Resilient Practical Approach

Kenneth A Yongabi

Phyto biotechnology Research Foundation (PRF), P.O.Box 921, Bamenda, Cameroon

*Corresponding Author: Kenneth A Yongabi, Phyto biotechnology Research Foundation (PRF), P.O.Box 921, Bamenda, Cameroon.

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Abstract

Oil spills from petroleum exploration constitutes a serious environmental damage and injury to climate that remains unresolved despite so much reports on bioremediation studies.Preliminary studies on the enhancement of biodegradation of used lubricating oil, a petroleum bye product, in soil amended with dried powdered Pleurotus tuberregium (PT) and Pleurotus ostreatus fruits (PO), Powdered sclerotium of Pleurotus tuberregium (SCPT) and spent substrate of Pleurotus ostreatus (SPO) was carried out in randomized pots of 400g of oil contaminated soil. The objective was to study the effect of mushroom and spent substrate as a biocatalyst in the cleanup of soil contaminated with petroleum products in the tropics. An absorption spectrophotometer with a wavelength of 640nm was used to analyze the oil content of the contaminated soil after defatting 50grams of it with a non-polar solvent-hexane. After seven weeks, the amount of lubricating oil in the untreated soil (not amended with the mushroom fruits, sclerotium and spent substrate) dropped from 960mg/g to 282mg/g. On application of varying quantities up to 60g each of PT, PO, SCPT, SPO, into the lubricating oil contaminated soil and left for seven weeks, the residual lube oil concentration dropped drastically from 960mg/g to 0.05mg/g, 0.5mg/g, 1mg/g and 27mg/g respectively. Pleurotus tuberregium supported faster break down of the oil than PO, SCPT and SPO. Corroboration between the crude protein and biodegradation efficiency was noted. PT (42.2%) protein while the least SCPO (9.8%) protein with least biodegradation. The PH of untreated soil (lubricating oil contaminated soil unamended) slowly dropped from 8.15 at the start of the experiment to 7.77 by the 7th week while PH of lubricating oil contaminated soil treated with PT, PO, SCPT, and SPO tend toward neutral (7.0) for the same period. Total aerobic mesophilic bacterial counts increased in the treated soil specimens from 1.5 x 104 CFu/g to too numerous to count (TNTC) in the 7th week. However, nutrient agar cultures of soil amended with mushroom fruits suggest luxuriant colonies/confluent and profuse growth than with sclerotium, spent substrate and control (unamended soil). More bacterial isolates and in higher counts were recovered from the amended soil including Pseudomonas spp, Bacillus spp, Enterobacter spp, Citrobacter, and E coli than with the control. The study suggests strongly that the biodegradation of the oil increased with increasing time, quantity and quality of biocatalyst added. The findings further suggest that mushroom and spent substrate of pleurotus ostreatus could be employed as cheap clean up agents of lubricating oil contaminated soil in the tropics and probably elsewhere.

Keywords: Soil, Oil, Mushrooms, Biocatalyst, Decontamination. Clean Up, Mini-Field Trial.

Introduction

The effect of oil spills on the environment is a subject that has triggered widespread concern globally. Oil and gas sector contributes significantly to global warming and distort ecosystems [1]. Climate is changing, population and global demands for oil and gas in increasing, yet the technologies for remediation of oil spills are in themselves unsustainable and expensive This is because oil spills adversely affect aquatic life and soil fertility [1]. For instance, Chindah and Braide reported that oil spills alter the biotic and abiotic profiles of water and soil. With increase in climate change induced floods and droughts, the likelihood of introducing new strains of organisms that might probably distort the autochthonous micro flora of soil and water, thus eliminating or reducing beneficial soil and aquatic microorganisms increases [2].

In sub–Saharan Africa at large, and in particular in Nigeria, Cameroon and Equatorial Guinea, a number of studies have been carried out on spills and biodegradation of crude oil and reported at regional level amongst others. These studies, which have largely focused on crude oil degradation with bacteria and micro fungi, are mostly on in vitro assessments [3-7]. Study efforts nowadays have concentrated on using metagenomics to enumerate and classify microbial communities involved in oil contaminated soil and ecosystems, while these studies are added value to fundamental research in environmental microbiology, the actual efforts in clean ups using climate adaptable technology requires acceleration.Reports on field or mini-field trials are rare and more so, the use of macro fungi such as mushrooms is rarer.

Studies from elsewhere revealed that a number of bacteria; *Pseudomonas, Bacillus, Acinetobacter, yeast; Candida, Saccharomyces and Moulds; Aspergillus, Mucor, Penicillium etc* have proven effective under Laboratory conditions in the degradations of crude oil. Unfortunately, the translation of these numerous findings to field benefits remains a feat. Yet, the ravage of oil spills especially in the Niger-Delta region of Nigeria; mechanic workshops and garages across Africa and probably in other parts of Asia and the world at large remains challenging and daunting [1]. Attention and research focus have to be titled toward simplification of techniques for easy adoption in the field. More so, little work in Nigeria and in sub–Saharan Africa at large, to the best of our knowledge has been reported on the breakdown of lubricating oil and more so employing mushrooms as biocatalyst [1]. There is an empirical observation that around mechanic workshops in Nigeria and in most African countries, where lubricating oils are used, the oil-contaminated soil remains bare for a long time due to the adverse effects of the oil. In this case, the soil sample was collected from a mechanic workshop where repairs and maintenance works are carried out only on passenger cars and light trucks of either gasoline or moderate duty engines.

However, it has been reported that motor oils for such gasoline engines and moderate duty engines, falls within the API: CC/SDM and ASTM: MIL-2104B specifications [8-10]. These categories of oils share an average main characteristic (Table 1), which agrees with the SAE viscosity grade for engine oils [11]. They also possess properties such as; extreme pressure protection for engines and hydraulic systems, resistance to oxidation and low wear and minimum deposits. They are also known to provide low engine temperature, anti-sludge and anti-rust performance respectively [8,12].

Table A: The Average Main Characteristics of Super Multi grade and Monograde oil for Naturally Aspirated Diesel Engines.

	SAE Viscosity Grade				
Characteristics	10W	20W50	30	40	50
Specific gravity at 15oC	0.880	0.890	0.890	0.895	0.900
Dynamic viscosity at –20oC, mPa.s (cPo)	3400	-	-	-	-
Dynamic viscosity at – 10oC, mPa.s (cPo)	-	4400	-	-	-
Kinematic viscosity at 40oC mn2/s (cSt)	34	170	113	149	230
Kinematic viscosity at 100oC, mn2/s(cSt)	6.1	18.5	12	45	19
Viscosity Index	120	122	95	95	95
Flash point, oC	220	250	230	240	250
Pour point,oC	-33	-27	-15	-15	-9

This study was, therefore, designed to explore and exploit the potential of *Mushrooms, Sclerotia and Mushroom* Spent substrate as agents/biocatalysts that could stimulate/speed up the activities of microorganisms in the breakdown of crude oil and oil contaminated soil.

Materials and Methods

Sample Collection and Processing

Four hundred grammes (400) of lubricating oil contaminated soil were introduced each into four plastic containers. Three of the containers were each treated with 40g, 50g and 60g of spent substrate of Pleurotus ostreatus, dried powdered fruits of Pleurotus osreatus. Another set up of four containers were each raised with the same treatment of 40g, 50g and 60g of dried powdered fruits of Pleurotus tuberregium and Sclerotium of Pleurotus tuberregium. One container each was left untreated and regarded as control. The twelve treated containers were randomized and the set up kept at 27oC (room temperature at the time of the experiment in Bauchi, Nigeria) for seven weeks. Every week for seven-week period, samples from each of the containers were taken for analysis of Residual lubricating oil content, PH analysis, and Microbial assays on the 7th week. The oil was extracted through a cold solvent extraction and oil qualified using U.V spectrophotometer. Replicate samples were used to conduct the experiment and mean values recorded.

Extraction of Lubricating Oil from Soil Samples

After each week fifty grammes of the lubricating oil contaminated soil was taken and 250mls of hexane was added, shaken vigorously and allowed to stand in a tight-fitting glass bottle. This was followed by gravity filtration using whatman filter paperNo.1. The oil-hexane extract was exposed in the sun for 5days for the hexane to evaporate. This same analysis was repeated weekly for 7weeks as biodegradation progressed. The absorbance of the oil (residual lubricating oil designated RLO) was taken at 640nm using spectronic 20 UV spectrophotometer.

Microbiological Analysis of Treated and Untreated Soil Samples

One gram of each of the soil samples were collected and suspended in 9mls of sterile distilled water in a 250ml beaker, the content was intermittently agitated to dislodge lumps and then filtered using whatman filter paper No 1. Appropriate serial four-fold dilutions were carried out and cultured on to Nutrient and MacConkey agars Oxoid Ltd, using the pour plate method. Each sample was cultured in duplicate and counts of visible colonies were taken with a colony counter after incubation at 37 degrees Celsius for 24 to 48 hours. Characterization of the isolates was employed using keys specified in Bergey, s Manual of Determinative Bacteriology reported in Cheesbrough*1984*. The colony structure, macromorphology as well as Micro morphology through gram-stained films were done, their specific identities were further elucidated using a panoply of biochemical tests, catalase, indole, Methyl Red Voges proskauer, Oxidase reaction and ability to ferment sugars, grown in urea broth and utilization of citrate were also tested.

Protein Analysis Determination using Keddah Nitrogen

One to two grams of air-dried fine powder together with a spatula tip of selenium reaction mixture were placed in Kjedahl flask and treated with 6ml concentrated sulfuric acid. The sample was then heated until it becomes colorless. After cooling, the solution was then transferred to a one litre round bottom flask and 25 ml 30 percent sodium hydroxide was added.

The distillation, titrimetric analysis was done and further steps carried out following protocols described by Kjedahl and AOAC methods. The obtained Nitrogen value was then multiplied by 6.25 to obtain an approximate protein value for the mushroom samples.

Soil Moisture Content Analysis

Initial weight of the soil go was taken using a field weighing balance. The sample was then dried in an oven at 60 degrees Celsius for 8 hours and allowed to cool. The samples were then kept in a desiccator for 24 hours and weight taken repeal until a constant weight was obtained cg.Percentage moisture is taken as the difference of the initial and final weight multiplied by a hundred percent and divided by the initial weight.

Table 1: Results of the U-V visible analysis of contaminated soil Sample using spent substrate of Pleurotus Ostreatus as BIOCATALYST

	O grams Biocata-lyst	40grams Biocata-lyst	50grams Biocata-lyst	60grams Biocata-lyst
BT (Weeks)	AbsRLO (A) (mg/g)	AbsRLO (A) (mg/g)	Abs.RLO (A) (g/g)	Abs RLO (A) (mg/g)
1	0.115 960	0.109 960	0.104960	0.099 960
2	0.111 880	0.100760	0.089579	0.077430
3	0.095 744	0.077338	0.057266	0.045 280
4	0.080 605	0.058 210	0.041 192	0.030 130
5	0.077570	0.030 110	0.02997	0.01173
6	0.069493	0.01182	0.019 69	0.008 63
7	0.059282	0.00978	0.017 46	0.005 27

BT→ Biodegradation Time (BT)

Abs→ Absorbance

RLO → Residual Lubricant Oil Content



Figure1: Fruited Pleurotus Ostreatus on Corn Cobs/ Sawdust.

1. Fruits
2. Spent Substrate

Table 2: Results of U-V Visible analysis of contaminated Soil Sample using Fruits of Pleurotus Ostreatus.

	O grams Biocata-lyst	40grams Biocata-lyst	50grams Biocata-lyst	60grams Biocatalyst
BT (Weeks)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs. RLO (A) (mg/g)
1	0.115960	0.103960	0.100 960	0.085960
2	0.111880	0.091 540	0.089380	0.080 350
3	0.095744	0.084195	0.047 310	0.03580
4	0.080605	0.020 70	0.020200	0.09851
5	0.077570	0.009 33	0.008 70	0.006 9
6	0.069 493	0.003 14	0.001 20	0.001 2
7	0.059 282	0.008 7	0.00038	0.00001 0.5

BT → Biodegradation Time

Abs. → Absorbance

RLO → Residual Lubricant Oil Content

Table 3: Result of UV- Visible Analysis of Contaminated Soil Sample using Sclerotium Powder of Pleurotus Tuberregium.

	O grams Biocata-lyst	40grams Biocata-lyst	50grams Biocata-lyst	60grams Biocatalyst
BT (Weeks)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs. RLO (A) (mg/g)
1	0.115960	0.103960	0.100 960	0.085960
2	0.111880	0.091 700	0.089589	0.080 406
3	0.095744	0.084427	0.047 342	0.0353 81
4	0.080605	0.020 301	0.020127	0.098100
5	0.077570	0.009111	0.008 60	0.006 31
6	0.069 493	0.00344	0.001 15	0.00111
7	0.059 282	0.00812	0.00033	0.00001 1

BT → Biodegradation Time

Abs. → Absorbance

RLO → Residual Lubricant Oil Content



Figure 2: Fruited Pleurotus Tuberregium on 1-Year-Old sclerotia.

1. Fruits
2. Sclerotia

N.B Sclerotium of pleurotus tuberregium has been used to clean wastewater.
Yongabi K.A. (2004). www.biotech.kth.se/kennetho4

Mushrooms have been used in anaerobic digestion as biocatalyst to speed up biogas production process and also as biocatalyst in the breakdown of used plastic bags. Yongabi, K.A 2005. Unpublished.

Table 4: Results of UV –Visible Analysis of Contaminated Soil Sample using Fruits of Pleurotus Tuberregium fr.Singer.

	O grams Biocata-lyst	40grams Biocata-lyst	50grams Biocata-lyst	60grams Biocatalyst
BT (Weeks)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs.RLO (A) (mg/g)	Abs. RLO (A) (mg/g)
1	0.115960	0.103960	0.100 960	0.085960
2	0.111880	0.091 595	0.089400	0.080 391
3	0.095744	0.091 595	0.047 194	0.035 93
4	0.080605	0.020 50	0.02040	0.09823
5	0.077570	0.00915	0.008 10	0.006 5
6	0.069 493	0.003 5	0.001 1	0.0010.4
7	0.059 282	0.0080.9	0.0003 0.5	0.0001 0.08

BT → Biodegradation Time

Abs. → Absorbance

RLO → Residual Lubricant Oil Content

Table 5: Crude Protein Analysis of Locally Harvested Pleurotus Tuberregium and Cultivated Pleurotus Ostreatus.

Mushroom specimen/type	Protein Content	Method used/Remarks
Pleurotus ostreatus	35% by dry weight	Spawn is exotic, but locally cultivated by K.A. Yongabi using 70% sawdust + 30% maize brand
Spent substrate (after 2nd flush)	9.8% by dry weight	Analysis using Kjedah's (AOAC) method.
Pleurotus tuberregium fr, singer	42.2% by dry weight	Fruited from Sclerotium (2yrs old) from Cameroon. Analysis using Kjedah's (AOAC) method.
Sclerotium of P. tuberregium	18.0% by dry weight	Analysis using Kjedah's method.

- Initial concentration of oil contaminant in soil 800mg/L
- Average environmental temperature 30oC at the time of experiment.
- Season when experiment was conductedDry season
- Initial soil PH uncontaminated soil 6.56
- Initial contaminated soil PH8.44
- Initial soil moisture content 16%
- Operating soil moisture content26%

(Experimental condition, Bauchi state meteorological station consulted for local weather data)

Table 6: Bacteria Analysis of Lubricating oil Contaminated Soil.

Parameter	Total aerobic Mesophilic counts
Initial bacteria/counts at the start of the experiment (lubricant oil contaminated soil as control)	1.5 X 104 cfu/g . Pseudommas sp . Citrobacter sp
Final bacteria counts on the 7th week treated with spent of PleurotusOstreatus	TNTC: Pseudomonas sp : E coli : Citrobacter sp : Bacillus sp : Enterobacter
Final bacterial counts on the 7th week treated with powdered Sclerotium of Pleurotus tuberregium	TNTC: Pseudomonas : E coli Citrobacter sp : Bacillus sp : Enterobacter
Final bacterial counts on the 7th week treated with dried fruits of pleurotus Ostreatus	TNTC* : Pseudomonas : E coli : Citrobacter : Bacillus : Enterobacter
Final bacterial counts on the 7th week treated with dried fruits of P tuberregium	TNTC* : Pseudomomas : E coli, Enterobacter : Citrobacter, : Bacillus

- Confluent Growth. TNTC = Too numerous to count.

Table 7: PH Variation with Bioremediation Time.

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Treatment with spent substrate of pleurotus Ostreatus	8.09	7.81	7.94	741	7.34	7.30	7.26
Treatment with Sclerotium of P tuberregium	8.09	7.7	7.7	7.5	7.32	7.31	7.28
Treatment with fruits of pleurotus Ostreatus	8.07	7.48	7.3	7.29	7.2	7.1	7.09
Treatment with fruits of pleurotus tuberregium	8.06	7.4	7.39	7.28	7.12	7.1	7.0
Control, untreated lubricant oil contaminated soil	8.15	8.08	8.02	7.01	7.92	7.87	7.77



Figure 3: The Potted Experiment in a Randomized Design, Aloe Vera Growing on the Treated Soil (Soil Was Contaminated with Lubricant Oil and Now Recovered and Recharged and Able to Support Crop).

Results and Discussion

The results of this preliminary study indicated that the spent substrate of Pleurotus Ostreatus (SPO), dried fruit powder of Pleurotus tuberregium (PT) dried fruit powder of Pleurotus ostreatus (PO) and sclerotium of Pleurotus tuberregium (SCPT) aided the degradation of lubricating oil in the soil. The results are presented in table 1 to 3. At the start of the experiment, the level of lubricating oil in the contaminated soil was 960mg/g at an absorbance of 0.115. At the end of the second week, the residual lubricating oil content of the contaminated soil had depreciated slightly to 760mg/g 579mg/g and 430mg/g after treatment with 40g, 50g and 60g of Pleurotus ostreatus spent substrate respectively. Compared to the untreated lubricating oil contaminated soil, at the end of the second week, the degradation was only 880mg/g. The absorbance decreased with increasing quantity of biocatalysts. Decreased absorbance in this experiment reflects increased biodegradation. Furthermore, at the end of the seventh week, the residual lubricating oil content has decimated to 27mg/g with a corresponding absorbance of 0.005. From the observation, it is evident that, biodegradation has taken place. The same trend was generally observable with lubricating oil-contaminated soil amended with dried powdered fruits of Pleurotus ostreatus and Pleurotus tuberregium as well as with Sclerotium of Pleurotus tuberregium. The residual lubricating oil content of the soil treated with 60 grams dried powdered fruits of Pleurotus ostreatus depreciated from 960mg/g to 350mg/g, 80mg/g, 51mg/g, 9mg/g, 2mg/g, and 0.5mg/gfor weeks 2, 3, 4, 5, 6, and 7 respectively.

As indicated earlier as a general trend, treatment using 60grams biocatalyst had the best biodegradation. For dried powdered fruits of Pleurotus ostreatus, the Residual Lubricating oil content at the end of the 7th week treated with 0g, 40 g, 50g and 60g was 282mg/g, 7mg/g, 8mg/g, and 0.5mg/g respectively. There is a significant difference in the Residual Lubricating oil contents judging from the results between the untreated sample and the treated samples and from an initial lubricating oil content of 960mg/g slashing down to 0.5g in the 7th week for Pleurotus ostreatus fruits depicts that with increased time the probability of complete utilization of the oil is not unlikely.

Pleurotus tuberregium exhibited better results that with Pleurotus ostreatus.The least performance, though far better than the control was observed with Spent Substrate of Pleurotus ostreatus.The high crude protein value of Pleurotus tuberregium fruits as obtained in this study, Table 5, may be responsible for the biodegradation efficiency. The least protein content was observed with the spent substrate of Pleurotus ostreatus and, apparently, least biodegradation was observed with the spent substrate of Pleurotus ostreatus as well. Nitrogen is a key energy source for microbial cell wall and the comparatively high nitrogen level must have supported prodigious proliferation of both microbial population and diversity as evidenced in the results. Furthermore, the high carbon source from the oil makes a good Carbon/Nitrogen level which is very favorable for microbial growth. The accompanying environmental conditions of temperature, moisture and PH must have played a supportive role. These conditions are vital for the growth and physiology of microorganisms and may have been absent in the initial lubricating oil contaminated soil. In tandem with this, Table 6 shows that the initial bacterial counts for the lubricating oil contaminated soil is low (15000cfu/g) with fewer species (Pseudomonas and Citrobacter)as opposed to treated soil samples where the bacterial counts were Too numerous to count(TNTC) with more bacterial species, Pseudomonas, Escherichia coli,Citrobacter,Enterobacter,Citrobacter,and Bacillus spp.Another interesting observation was the recovery of luxuriant and large colonies on nutrient agar from samples treated with Pleurotus tuberregium.The possible effect of the high level of nitrogen as exemplified in the protein content is not unlikely, Table 5.

The PH of untreated soil (Lubricating oil contaminated soil unamended) slowly dropped from 8.15 at the start of the experiment to 7.77 by the 7th week while PH of lubricating oil contaminated soil treated with PT, PO, SCPT, and SPO tend toward neutral for the same period. It, thus, can be generalized that the more the amount of biocatalyst added to the oil contaminated soil, the quicker the degradation. There are indications that the mushroom samples may have triggered the multiplication, growth and activity of aerobic mesophilic bacterial (microbial) counts. Their involvement in the utilization of the lubricating oil in the soil is suspected. The results does not show that neither Pleurotus Ostreatus spent substrate,Pleurotus ostreatus fruits,,Pleurotus tuberregium,nor Sclerotium of Pleurotus tuberregium are responsible for the clean-up but that they probably supply the needed nutrients to support microbial activity which in turn utilizes the oil at a faster rate.

Besides, the isolation of bacteria like Pseudomonas spp, Enterobacter and Bacillus spp further confirm bacterial involvement in the degradation. These organisms are widely implicated in the clean-up of oils [13,14]. For example, in the previous studies of K0 and Leaeault, two strains of Pseudomonas aeruginosa K1 were isolated from gasoline – polluted soil and were able to grow on n alkanes and pristanes [14]. Most of the isolates were aerobes, and a number of aerobic bacteria have been isolated in previous studies including beta proteobacteria and common soil bacteria such as Burkholderia cepacia and Burholderia solanacearum. Admittedly, a number of biodegradation activity have taken place under anaerobic conditions with anaerobes playing a key role. Anaerobic degradation of benzene as well as aromatic hydrocarbons have been reported Previous studies have observed that many of the soil inhabiting or plant pathogenic fungi such as Trichoderma, Aspergillus, paecilomyces, Penicillium, Botrytis and Mucor all produce organic acids as primary means of attack on organic and inorganic materials [15,16]. There is a possibility from that mushroom extracts may even help speed up anaerobic degradation process as revealed in this study.

The technique of using nutrients as shown in this study, in oil-contaminated fields, may solve the problem of resistance –for instance- cyclo –alkanes have been observed as the most resistant molecules to microbial attack among saturated hydrocarbons and these are major component of “drilling oils” [17]. These compounds (oils and industrial solvents) have a widespread occurrence and are now increasingly resistant to microbial attack. Reports equally exists elsewhere on the employment of mixed cultures of selected hydrocarbon degrading bacteria to increase biodegradation but these might be expensive and requires a lot of expertise in like the use of these biocatalysts as observed in this study. Finally, from the results of these studies, the PH of the lubricating oil contaminated soil showed a decreasing trend as biodegradation process increases. This also tallies with the fact aerobic bacteria counts increased implying that the PH has changed to favor their activity [18-29].

To Cap it all, Take Home Lessons from this Preliminary Study are:

- Mushrooms and spent substrate of Pleurotus ostreatus provide nutrients that support microbial break down of oil contaminated soil in the tropics and above up faster clean up and better soil recharge than with just the microorganisms alone. This could be comparatively cheaper than always trying to use and depend on pure cultures as the case has been
- Pleurotus tuberregium a tropical mushroom is a better catalyst for the decontamination of lubricating oil-contaminated soil than spent compost of Pleurotus ostreatus. From the findings of this study,iIt is possible to employ mushroom fruits,sclerotium,and spent compost of Pleurotus ostreatus as a practical solution to clean up lubricating oil contaminated soil and regain vegetation.

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