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Evaluating the Mycoremediation Potential of Mushroom Species *Lentinus subnudus* and *Calocybe indica* in Crude Oil-Contaminated Soils

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ABSTRACT

Crude oil pollution severely impacts the chemical, biological, and physical properties of agricultural soil. This study, evaluated the bioremediation potential of Lentinus subnudus and Calocybe indica mushrooms in decontaminating crude oil-polluted soil from Ahoada East (AES), Rivers State, Nigeria. Soil samples from contaminated and uncontaminated sites served as experimental and control groups, respectively. Using a 2x2 factorial arrangement in a Completely Randomized Design, replicated three times, the experiment involved overlaying 200 grams of soil samples with 100 grams of mushroom spawn and 250 grams of sterile sawdust, with data collected at four-week intervals for three months. Analysis revealed that both mushroom species significantly reduced Polycyclic Aromatic Hydrocarbons (PAHs). The Lentinus subnudus decreased Benzo(G,H,I)Perylene by 77.60% at week 4 and Benzo(B)fluoranthene by 50.06% at week 12, while Calocybe indica achieved reductions of 96.37% and 52.62%, respectively. Heavy metal residues in the soil were also reduced, with Lentinus subnudus decreasing CU residue by 25.13% and Calocybe indica by 78.94% at week 12. The bacterial population increased by 95.83% and 36.14% at weeks 8 and 12 in dilution 3 with Calocybe indica, while fungal population increased by 41.25% at week 8 using Lentinus subnudus. The PAH-utilizing bacteria decreased by 62.5% at week 8 with Calocybe indica. Both mushrooms effectively mycoremediated contaminated soils, with Calocybe indica showing more consistency than Lentinus subnudus.

Keywords: Mycoremediation, crude-oil contaminated soil, fungi, mushroom, polycyclic aromatic hydrocarbon

INTRODUCTION

A significant global environmental concern exacerbated by urbanization and industrialization is the contamination of soil and other terrestrial resources by crude oil and petroleum products (Tripathi *et al*., 2015; Henry *et al.*, 2018). These contaminants are particularly challenging to manage due to their stability and durability in the environment (Song *et al.*, 2021; Uddin *et al.*, 2021). Dadrasnia and Agamuthu (2013) estimate that approximately 8.8 million metric tons of oil reach aquatic environments annually. However, the majority of oil spills, whether in marine or terrestrial ecosystems, result from human activities. This pollution poses a serious threat to ecosystems and human health, sparking extensive research efforts aimed at finding innovative and cost-effective solutions to mitigate its impact on soil entity (Bala *et al*., 2022).

The primary sources of petroleum hydrocarbons and related byproducts in soil are unintentional crude oil spills, fuel pollution, refining activities, and poorly managed transport and distribution service of petroleum products (Grifoni *et al*., 2020). Crude oil and petroleum-based contaminants mainly consist of hydrocarbons—molecules composed of hydrogen and carbon.

These hydrocarbons, due to their hazardous components, negatively affect the chemical, physical, and biological properties of soil (Grifoni *et al*., 2020; Haider *et al*., 2021; Mirjani *et al*., 2021; Adetunji *et al*., 2021; Chokor, 2021). According to Czarny *et al*. (2020) and dos Santos and Maranho (2018), capillary action and gravity further exacerbate the spread of these pollutants through vertical movement in the soil profile. Consequently, crude oil pollution disrupts essential microbial and enzymatic processes that are crucial for maintaining soil fertility and agricultural productivity. Moreover, the presence of benzene compounds, particularly polycyclic aromatic hydrocarbons (PAHs), introduces additional threats, as these chemicals are toxic and unfriendly to cropping, thus classified as priority pollutants (Vijayanand *et al*., 2023; Idris *et al*., 2019).

Conventional remediation methods—such as chemical dispersion, burning, and mechanical removal are often ineffective, costly, or hazardous to the environment. Therefore, there is an urgent need for environmentally sound and sustainable soil remediation techniques. Bioremediation, which involves the use of living organisms to break down, modify, or remove pollutants from soil has shown significant promise. Beyond bacteria and fungi, research has also highlighted the potential of certain plants in phytoremediation (Raklami *et al.,* 2022; Mocek-Płóciniak *et al.,* 2023). Specifically, the use of mushrooms in myco-remediation has gained attention, with Adenipekun and Lawal (2012) and Mohamadhasani and Rahimi (2022) exploring the efficacy of fungal mycelium for pollutant degradation. In particular, white rot fungi have demonstrated substantial potential in breaking down complex pollutants through the production of extracellular enzymes such as laccases, manganese peroxidases, and lignin peroxidases. These enzymes enable fungi to degrade a wide array of contaminants, often more efficiently than bacterial or other fungal methods (Yadav *et al.,* 2021). Filamentous fungi, in particular, have been extensively studied for their ability to degrade aliphatic hydrocarbons and PAHs, utilizing these organic pollutants as carbon sources (Goutam *et al.,* 2021).

Building on this existing body of knowledge, our study investigates the potential of *Calocybe indica*, a largely unexplored species of milky mushroom, for myco-remediation of crude oil-contaminated soil. This work represents the first attempt to utilize *Calocybe indica* in both bioremediation and phytoremediation. Previous studies have focused on other species, such as the edible mushroom *Lentinus subnudus* (commonly known as white rot fungi), but the aim was to compare the intrinsic myco-remediation capacities of *Calocybe indica* and *Lentinus subnudus* in this study. Both mushroom species were applied to crude oil-contaminated soil from Ahoada East (AES) in Rivers State. The findings from this comparative study offer valuable insights into the role of edible mushrooms in soil remediation efforts, particularly in addressing petroleum hydrocarbon pollution.

MATERIALS AND METHODS

Study area and experimental set up

The soils for this study were sampled from crude oil contaminated and non-contaminated sites in Ahoada East Local Government Area, situated on 5°05'N and 6°39'E, Rivers State. All soil samples were taken at 0 – 20 cm and were moved to the Soil science laboratory at Babcock University to set up a 2 x 2 factorially arranged completely randomized design (CRD) experiment, which was replicated three times. The treatment combination included 2 factors and factor one comprised of 2 levels: control (non-contaminated soil) and crude oil-contaminated soil, while factor two was made up of two test mushrooms—*Lentinus subnudus* and *Calocybe indica.*

Soil and plant analyses

For the following physical and chemical analyses, soil samples were suitably sieved and allowed to air dry. The hydrometer method (Bouyoucos, 1962; Agbenin, 1995) was used to analyze the particle size distribution of the soil. The potassium dichromate method was used to assess the organic carbon content of the soil and the mushrooms (Walkey and Black, 1934). Using a pH meter set to a 1:1 soil solution ratio, the pH of the soil and the mushrooms were measured (Thomas, 1966). By the Bray 1 extraction, the amount of available phosphorus in the soil was calculated (Murphy and Riley, 1972). Using 1N ammonium acetate (pH = 7), exchangeable bases in soil were identified (Okalebo *et al*., 1993). While calcium (Ca) and magnesium (Mg) were measured using AAS, potassium (K) and sodium (Na) were assessed using flame photometry. 1N KCl was used to calculate exchangeable acidity (Al^{3+} and H^{+}).

Bioremediation (myco-remediation) set up and evaluation of the effect of Polycyclic Aromatic Hydrocarbon (PAH) on bacterial and fungi population

Tissue culture of the freshly collected mushroom was prepared by cutting the tip of the stipe after removing the pileus and placed on sterilized Potato Dextrose Agar (PDA) medium. This was carried out under sterile conditions in a laminar airflow in the soil science laboratory at Babcock University. The pure cultures of isolates were maintained on sterilized potato dextrose agar (PDA) slants and subsequently transferred to PDA plates and incubated at 25°C to obtain good mycelial growth. This was later used for mushroom spawn formation using suitable standard substrates. Two hundred and fifty grams of crude oil-contaminated soil and non-contaminated soil samples were transferred into cleaned and dried 475-ml mayonnaise bottles. Two hundred grams of sterile sawdust and hundred grams each of the mushroom spawns were used to overlay the soil samples using the modified version of (Adenipekun and Lawal, 2012). Non-contaminated soil was used as the control experiment with respect to polycyclic aromatic hydrocarbon utilizing bacterial and other bacterial species present in comparison to those in crude oil-contaminated soil. Nutrient Agar and Potato Dextrose Agar were used for isolation of general bacterial and fungi while Mineral Salt Agar (MSA) and Bushnell-Haas Agar were used for isolation of polycyclic aromatic hydrocarbon utilizing bacterial through microbial count method.

Evaluation of the biodegradation rate of polycyclic aromatic hydrocarbon in contaminated myco-remediated soils

Contaminated soil samples which had been exposed to myco-remediation from 0 to 12 weeks were extracted using a modified methanol-chloroform-water method and analyzed by GC-MS. Extracts were treated with methanol and evaporated under N2. A Shimadzu GCMS-QP 2010SE equipped with an MDV-5 capillary column analyzed the samples at 0, 4, 8, and 12 weeks. Helium was used as the carrier gas. Polycyclic aromatic hydrocarbon metabolites were identified based on 90% similarity between MS spectra of unknowns and reference standards. Semiquantitative kinetics analysis (Co-Ct/Co × 100) was used to assess the myco-remediation rate of polycyclic aromatic hydrocarbon at different weeks in contaminated soil from Ahoada east LGA, adhering to the modified version of Momoh *et al.* (2020).

Data collection and statistical analysis

Data were collected from the microcosm setup at 2 weeks interval for 3 months in both contaminated and non-contaminated soils to check the level of myco-remediation that was achieved by either of the two species of mushroom which were used. Data collected was subjected to analysis of variance using R (version 4.3.2, 2023), while means were separated using 5% significance level based on the independent t-test.

RESULTS AND DISCUSSION

Table 1 presents the physical and chemical properties of non-contaminated and contaminated soils. A significant difference was observed in pH between the control soil and the contaminated soil. The pH of the control soil was moderately acidic (5.4), whereas the pH of the contaminated soil was closer to neutral (7.1). Additionally, soil organic carbon in Ahoada East L.G.A contaminated soil was significantly higher (21.0 g/kg) compared to the non-contaminated soil, which recorded only 2.0 g/kg.

Table 1: Physical and chemical properties of non-contaminated and contaminated soils

Parameter	Unit	Control	Contaminated	T-value
pH		5.4b	7.1a	0.02
TN	g/kg	0.1	0.1ns	1.01
Av.P	mg/kg	5a	3.7b	0.03
OC	g/kg	2.0b	21a	0.006
MC	g/kg	118	113ns	0.07
Sulphur	mg/kg	1.1a	0.5b	0.01
Bulk density	g/cm^3	1.6	1.4ns	0.12
EC	(us/cm)	95b	458a	0.002
Sand	g/kg	750b	910a	0.021
Clay	g/kg	190a	70b	0.003
Silt	g/kg	60a	20a	0.001
Ca	mg/kg	496.7b	1092.8a	0.0001
Mg	mg/kg	416.7	494.3ns	0.052
K	mg/kg	292.5	270.6ns	0.056
Na	mg/kg	42	41ns	0.131
Mn	mg/kg	55.1	55.4ns	0.666
Pb	mg/kg	2.0	1.7ns	0.117
Ni	mg/kg	3.5b	21.4a	0.003
Cr	mg/kg	0.12b	0.98a	0.024
Co	mg/kg	3.4b	29.3a	0.008
Cd	mg/kg	1.5b	11.4a	0.001

Note: Means bearing different letters row-wise are not statistically different at 5% significance level based on the independent t-test and ns = non-significance accordingly.

These findings suggest that the presence of crude oil in soil considerably modifies its pH, organic carbon content and a few heavy metals. The pH recorded is different from that of other studies that found a lower soil pH (Wang *et al*., 2013; Devatha *et al.*, 2019) which is likely due to soil types, and contamination sources variations. Because of the presence of hydrocarbon molecules, the higher soil organic carbon in contaminated soils is consistent with expectations, underscoring the influence of crude oil contamination on soil characteristics.

Table 2 presents the physical and chemical properties of the two mushroom species used for mycoremediation. It was observed that *Calocybe indica* had significantly higher moisture, Fe and Cu content by 20.33%, 33.02% and 22.77% respectively compared to *Lentinus subnudus*.

Additionally, both species exhibited a relatively high organic carbon content, although that of *Calocybe indica* was numerically lower (2.685) than *Lentinus subnudus* (2.837). Mushrooms, such as *Calocybe indica*, produce critical extracellular enzymes like laccases, manganese peroxidases, and lignin peroxidases, which play a pivotal role in the degradation of polycyclic aromatic hydrocarbons (PAHs). The high moisture content in the substrate is essential for maintaining the enzymatic activity of these fungi, ensuring efficient degradation of complex hydrocarbons such as PAHs. In *Calocybe indica*, the increased moisture also promotes fungal growth by facilitating the expansion of the mycelial network, thereby improving surface contact with contaminants. This enhanced contact maximizes the mushroom's capacity to metabolize or sequester PAHs, thus improving its remediation efficiency (Asgher *et al*., 2014).

Table 2: Physical and chemical properties of the two mushroom species used for mycoremediation

Sample	pH	TN	OC	MC	Fe	Cu	Zn
Unit		(g/kg)		(%)	(mg/kg)		
Calocybe indica	5.33	1.945	2.685	51.946	45.555	4.366	21.427
Lentinus subnudus	5.72	1.972	2.837	41.387	30.511	3.377	22.363
T-value	0.45	0.89	0.62	3.48	4.77	0.58	1.35

In essence, the findings reveal that *Lentinus subnudus* and *Calocybe indica* have difference in some of the measured chemical and physical characteristics, which may have an implication on their myco-remediation efficacies. *Calocybe indica's* higher level of moisture increases its potential to attract additional water molecules by Van der Waals force during the myco-remediation, to effectively interact with the soil pollutants. Both *Lentinus subnudus* and *Calocybe indica* have appreciable levels of organic carbon that enable fungal growth and enzymatic activity, which are essential for bioremediation processes. These results highlight the importance of studying the characteristics of the mushroom species employed in myco-remediation techniques, for effective soil pollution remediation strategy.

Table 3 illustrates the effectiveness of *Lentinus subnudus* and *Calocybe indica* in reducing the concentrations of specific polycyclic aromatic hydrocarbons (PAHs) in the contaminated soil over different time intervals. *Lentinus subnudus* was able to reduce the concentration of benzo(B)fluoranthene by 50.06% at week 12, while *Calocybe indica* achieved reductions of 67.62% at week 8 and 52.62% at week 12 for the same PAH compound. For Dibenz(A,H) Anthracene, *Lentinus subnudus* reduced the concentration by 67.23% at week 4, while *Calocybe indica* consistently reduced the concentration of the PAH compound by 87.21% at week 8 and 76.44% at week 12. Additionally, *Lentinus subnudus* decreased Indeno(1,2,3-CD) Pyrene by 17.68% at weeks 8, whereas *Calocybe indica* achieved higher reduction by 51.20% at week 8. Furthermore, while *Lentinus subnudus* showed substantial reductions in concentration of Benzo(G,H,I) Perylene by 77.60% at week 4 and 78.36% at week 8, *Calocybe indica* demonstrated more consistent reductions of 96.37%, 43.23%, and 32.88% at weeks 4, 8, and 12, respectively.

The results from this study demonstrate the significant capacity of both *Lentinus subnudus* and *Calocybe indica* to effectively reduce PAH concentrations in contaminated soil through myco-remediation. The observed differences in PAH reduction rates between the two mushroom species highlight the potential influence of their inherent characteristics, such as moisture content and organic carbon components. The *Calocybe indica*, with its higher moisture content and comparable organic carbon levels, may have exhibited more consistent and effective PAH degradation capabilities compared to *Lentinus subnudus.* This aligns with previous studies suggesting that mushroom species with specific enzymatic profiles, including cellulases, lignases, and peroxidases, play crucial roles in PAH degradation (D'Annibale *et al*., 2005; Adenipekun and Lawal, 2012; Min *et al*., 2015). These results corroborate previous findings, which emphasize the efficacy of myco-remediation in reducing PAH concentrations in contaminated soils (Okparanma *et al.*, 2011; Yadav *et al.*, 2021; Kechi-Nwogu and Akpan, 2022). The higher effectiveness of *Calocybe indica* over *Lentinus subnudus* in PAH reduction underscores the importance of selecting suitable mushroom species based on their biochemical capabilities for effective soil bioremediation strategies. **Table 4** details the myco-remediation of heavy metals in crude oil-contaminated soil from Ahoada East using *Lentinus subnudus* and *Calocybe indica* at different time intervals. At week 4, Lentinus subnudus significantly reduced Copper (Cu) residue by 48.09% compared to baseline, whereas Calocybe indica achieved a reduction of 42.38% for the same heavy metal. By week 12, *Lentinus subnudus* reduced Cu residue by 25.13%, while *Calocybe indica* showed a more substantial reduction of 78.94%. Similarly, *Lentinus subnudus* significantly reduced Zinc (Zn) residue by 75.13%, and *Calocybe indica* reduced Zn residue by 75.11%.

Table 3: Myco-remediation of polycyclic aromatic hydrocarbon in contaminated soil using *Lentinus subnudus* and *Calocybe indica* at different weeks

		CLS	CCI	CLS	CCI	CLS	CCI
Parameters	WK0	WK 4		WK 8		WK 12	
BENZO(B)FLUORANTHENE	0.211771	2.66744	2.90192ns	3.58439a	0.939614b	1.33196	1.37486ns
T-Value		0.922		1.55e-04		0.785	
CHRYSENE	0.178076	2.15195b	3.18674a	4.47515	4.49961ns	0.382937b	2.59319a
T-Value		1.33e-05		1.002		2.44e-09	
BENZ(A)PYRENE	0.278522	0.985705b	1.68488a	0.609578b	4.40481a	1.09273a	0.397355b
T-Value		1.66e-03		3.57e-06		2.99e-04	
DIBENZ(A,H)ANTHRACENE	1.02312	0.3354a	0.13087b	7.29659a	2.19994b	2.57692a	0.241065b
T-Value		1.5e-03		2.0e-05		4.17e-08	
INDENO(1,2,3-CD) PYRENE	0.206658	0.127815a	0.03839b	1.04684a	0.12024b	0.17077b	0.226141a
T-Value		3.11e-05		2.45e-10		4.11e-02	
BENZO(G,H,I)PERYLENE	0.57604	0.129023b	0.02087b	0.124645b	0.336047a	0.724193a	0.393487b
T-Value		5.29e-07		4.3e-04		4.55e-02	

Note: CLS = contaminated soil + *Lentinus subnudus* and CCL = contaminated soil **+** *Calocybe indica*. Mean values were compare across rows and therefore means for each parameter under a given week with different letters are statistically different at 5% level of significance using an independent T-test.

Table 4: Mycoremediation of Zinc and Cupper in contaminated oil using *Lentinus subnudus* and *Calocybe indica* at different weeks

Soil type	WK0	WK 4 (LS)	WK 4 (CI)	WK 12 (LS)	WK 12(CI)
			Zn(mg/kg)		
Contaminated	505.5a	0.0066a	0.0093a	125.7a	125.8a
Control	8.4b	0.0005b	0.0020b	32.3b	29.8b
T-Value	3.3ie-11	5.0e-5	1.7e-06	5.23e-08	8.44e-11
Soil type					
			Cu(mg/kg)		
Contaminated	110.5a	57.2a	63.5a	82.5a	23.2
Control	7.4b	2.7b	3.6b	4.5b	3.9
T-Value	1.13e-09	8.91e-16	7.5e05	1.33-15	9.3e-18

Note: LS= *Lentinus subnudus,* CI = *Calocybe indica*. For each parameter the comparison was done across treatment, hence means across column with different letters are statistically different at 5% significance level using independent t-test.

The findings demonstrate the effectiveness of *Lentinus subnudus* and *Calocybe indica* in myco-remediating heavy metals from crude oil-contaminated soil. The differences observed in heavy metal reduction rates between the two mushroom species suggest variations in their bioaccumulation and enzymatic capabilities. *Calocybe indica's* superior performance in reducing Cu and Zn residues, especially evident at week 12, underscores its potential as a robust candidate for heavy metal myco-remediation. This aligns with previous studies highlighting the ability of edible mushroom species to serve as effective biosorbents for heavy metals in both aquatic and terrestrial environments (Jibran and Milsee, 2011; Luo *et al.,* 2013). Results from this study are consistent with the findings of Njoku *et al.* (2016), who reported similar reductions in heavy metals through myco-remediation of petroleum hydrocarbon-polluted soils using P. pulmonarius. Additionally, Ogbeide and Henry (2024) documented reductions in copper residues following mycoremediation, further supporting the efficacy of fungal-based remediation strategies. The substantial reduction in heavy metal residues observed in our study highlights the potential of *Lentinus subnudus* and *Calocybe indica* as eco-friendly alternatives for mitigating heavy metal pollution in contaminated soils.

Table 5 illustrates the impact of mycoremediation on bacterial populations in contaminated soil. Using *Lentinus subnudus,* we observed a significant increase in bacterial population by 34.41% at week 4 in dilution 4 of contaminated soil. Conversely, using *Calocybe indica*, bacterial populations significantly increased by 95.83% at week 8 in dilution 3, and by 36.14% at week 12 in contaminated soil. **Table 6** shows a notable decrease of 62.50% in hydrocarbon-utilizing bacteria in contaminated soil at week 8 after mycoremediation using *Calocybe indica* in dilution 4. This reduction can be attributed to the decreased availability of PAH compounds, which serve as carbon sources for these bacteria. No significant difference was observed in dilution 4 when *Lentinus subnudus* was used for mycoremediation at week 8 regarding hydrocarbon-utilizing bacteria.

These results indicate that mycoremediation using *Lentinus subnudus* and *Calocybe indica* significantly influences bacterial populations in crude oil-contaminated soil. The observed increases in bacterial populations post-mycoremediation suggest a potential stimulation of microbial activity, possibly due to the introduction of organic matter and enhanced nutrient availability following mushroom degradation activities. The significant decrease in hydrocarbon-utilizing bacteria after mycoremediation with *Calocybe indica* aligns with previous studies highlighting the role of fungal remediation in reducing microbial populations reliant on hydrocarbon substrates (Idowu and Ijah, 2018). This reduction is crucial as it indicates a potential shift in microbial community dynamics towards a less contaminated state, enhancing soil health and fertility. The observations from this study are consistent with research by Medaura *et al.* (2021), who similarly observed higher counts of aerobic heterotrophic hydrocarbon-utilizing bacteria in diesel oil-polluted soil compared to unpolluted soil. This underscores the ecological impact of hydrocarbon pollutants on microbial communities and supports the efficacy of mycoremediation in mitigating their effects. Our study also highlights the dual benefits of *Lentinus subnudus* and *Calocybe indica* in not only reducing contaminant levels but also in promoting beneficial shifts in microbial populations crucial for soil ecosystem recovery.

Table 7 illustrates the impact of mycoremediation on fungal populations in contaminated soil. Using *Lentinus subnudus*, we observed a significant increase in fungal population by 41.25% at week 8 in dilution 3 of contaminated soil compared to the fungal population in control soil. Conversely, fungal populations in contaminated soil after mycoremediation using *Calocybe indica* showed no significant difference from the fungal population in control soil at week 12. The results obtained demonstrate the differential effects of *Lentinus subnudus* and *Calocybe indica* on fungal populations during myco-remediation of contaminated soil. The significant increase in fungal population observed with *Lentinus subnudus* suggests a stimulatory effect of this species on fungal proliferation in response to the presence of crude oil contaminants. This increase in fungal biomass could contribute to enhanced organic matter decomposition and nutrient cycling in the soil, thereby promoting soil health and fertility. In contrast, the stability of fungal populations observed after mycoremediation with *Calocybe indica* indicates a potential equilibrium in fungal community dynamics. While this species may not stimulate fungal growth to the same extent as *Lentinus subnudus,* its effectiveness in reducing contaminants and supporting microbial communities, as seen in previous sections, underscores its role in soil remediation. These findings align with previous studies highlighting the role of fungal remediation in shaping microbial communities and enhancing soil resilience (Idowu and Ijah, 2018; Medaura *et al*., 2021). The observed variations in fungal population dynamics between *Lentinus subnudus* and *Calocybe indica* further emphasize the importance of species-specific interactions with contaminants and soil microenvironments.

Table 5: Effect of myco-remediation of contaminated soil on bacterial population within the soil

Soil Type	WK 0	WK 0	WK 4		WK 4		WK 8		WK 8		WK 12		WK 12	
			LS	CI	LS	CI	LS	CI	LS	CI	LS	CI	LS	CI
	(10^3 cfu/g)	(10^4 cfu/g)	(10^3 cfu/g)		(10^4 cfu/g)		(10^3 cfu/g)		(10^4 cfu/g)		(10^3 cfu/g)		(10^4 cfu/g)	
Contaminated	2.1	2.3	0.8b	3.4a	6.1b	2.1	1.1	1.5b	6.5	1.7	1.6	4.2a	9.4	5.3b
Control	2.4	1.9	1.2a	0.2b	9.3a	1.8	1.2	36.0b	6.5	2.2	1.3	1.3b	8.0	8.3a
T-Value	0.0692	0.056	0.037	4.2e-5	2.3e-04	0.11	0.09	1.2e-06	0.23	0.17	0.053	0.01	0.07	1.2e-4

Note: LS = *Lentinus subnudus,* CI = *Calocybe indica*

Table 6: Effect of myco-remediation of contaminated soil on hydrocarbon utilizing bacterial population within the soil

Soil type	WK0	WK0	WK4		WK4		WK8		WK8		WK12		WK12	
			LS	CI	LS	CI	LS	CI	LS	CI	LS	CI	LS	CI
	(10^{-3} cfu/g)		(10^3 cfu/g)		(10^4 cfu/g)		(10^3 cfu/g)		(10^4 cfu/g)		(10^3 cfu/g)		(10^4 cfu/g)	
Contaminated	2.6	0.9b	1.5	1.7	4.8b	1.3	8.8	1.8b	8.1a	0.9	1.5	1.3	7.9	7.8
Control	3.1	2.2a	1.2	1.7	7.6a	1.2	9.2	2.5a	5.3b	1.2	1.7	1.1	6.4	8.0
T-Value	0.08	1.2e-03	0.06	0.19	2.1e-04	0.05	0.05	4.1e-04	3.1e-03	0.08	0.06	0.05	3.2e-05	0.07

Note: LS = *Lentinus subnudus,* CI = *Calocybe indica*

Table 7: Effect of myco-remediation of contaminated soil on fungi population within the soil

Soil type	WK 0 (10^3 cfu/g)	WK 0 (10^4 cfu/g)	WK4 (10^3 cfu/g)		WK4 (10^4 cfu/g)		WK8 (10^3 cfu/g)		WK8 (10^4 cfu/g)		WK12 (10^3 cfu/g)		WK12 (10^4 cfu/g)	
			LS	CI	LS	CI	LS	CI	LS	CI	LS	CI	LS	CI
Contaminated	1.7b	3.7	6.3a	7.0b	5.7a	5.3b	8.0a	5.0b	5.3a	3.7b	7.3a	5.0b	5.7a	5.9
Control	2.7a	4.0	5.0b	8.0a	4.3b	6.0a	4.7b	6.7a	3.0b	5.3a	4.3b	7.3a	3.0b	6.0
T-Value	4.2e-02	0.12	3.3e-03	4.5e-04	3.1e-06	4.1e-03	3.8e-04	4.5e-07	2.8e-04	3.6e-07	3.1e-04	1.9e-02	2.2e-03	0.09

Note: LS = *Lentinus subnudus* and CI = *Calocybe indica*

CONCLUSION

From this study, it was observed that *Lentinus subnudus* (LS) demonstrated significant efficacy in the myco-remediation of polycyclic aromatic hydrocarbon compounds. However, *Calocybe indica* (CI) exhibited greater consistency in that regards across the study period compared to LS. Notably, CI also showed consistent effectiveness in myco-remediation of heavy metals, highlighting its robust remediation capabilities. Furthermore, CI facilitated a notable increase in bacterial populations post-myco-remediation, indicating its potential to enhance microbial activity and soil health recovery. In contrast, LS promoted a greater increase in fungal populations after myco-remediation, suggesting differential effects on microbial community dynamics. These findings underscore the distinct remediation potentials of LS and CI in crude oil-contaminated soils. While both species contribute significantly to soil bioremediation, CI consistency in reducing contaminants and promoting beneficial shifts in bacterial populations positions it as a promising candidate for sustainable soil remediation practices. Overall, this study enhances our understanding of the ecological roles of mushrooms in soil bioremediation and emphasizes the importance of species-specific approaches in optimizing remediation strategies for contaminated environments.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest on the information presented in this article.

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