*Environmental Toxicology and Chemistry*, 2025, 1–10
https://doi.org/10.1093/etojnl/vgae078
Advance access publication: January 6, 2025
**Original Article**

## Remediation and Restoration

# Mycoremediation of Louisiana sweet crude oil with *Pleurotus ostreatus* and nutrient amendments

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## Abstract

Oyster mushrooms (*Pleurotus ostreatus*) are known to metabolize polycyclic aromatic hydrocarbons in petroleum crude oil, making them candidates for oil spill remediation studies. This work aimed to assess *P. ostreatus* for its hydrocarbon degradation potential in estuarine conditions. In vitro experiments evaluated nutrient amendments based on *P. ostreatus* carbon: nitrogen: phosphorous (C: N: P) ratios to optimize mycelium growth, increase degradation efficiencies, and reduce potential nutrient runoff in broader applications. Image analysis was used to track mycelium growth response to various salinities, nutrient amendments, and oil types. Subsequent evaluation of alterations within the saturate, aromatic, resin, and asphaltene (SARA) fractions constituting Louisiana sweet crude (LSC) was conducted via SARA analysis. Results indicate that *P. ostreatus* mycelium tolerates estuarine salinities, with maximum growth between 5 and 15‰. Relative to 0‰, growth was reduced at salinities >25‰, but positive growth was still observed. Nutrient amendments significantly increased growth over 7 days relative to untreated samples ($p < 0.0001$). The combination of ammonium chloride and potassium phosphate yielded optimal mycelium growth after 7 days. Mycelium and nutrients significantly altered saturate ($p = 0.0015$) and asphaltene ($p = 0.0124$) fractions in LSC. This study suggests that mycelium growth is viable under estuarine salinities and can be enhanced with nutrient amendments. Introducing nutrient factors was shown to influence oil degradation. Results also indicate that mycelium can reduce recalcitrant oil fractions. Thus, this study highlights the adaptability of *P. ostreatus* to estuarine conditions and its response to nutrient amendments, all while offering a promising approach to crude oil bioremediation.

**Keywords:** bioremediation, polycyclic aromatic hydrocarbons, oil spills

## Introduction

Estuarine ecosystems contain vital habitats that act as natural buffers against storm surges and coastal erosion, provide water filtration, and serve as crucial nurseries for recreationally and commercially important species (Barbier et al., 2011). Petroleum pollution can severely impact these environments because compounds such as polycyclic aromatic hydrocarbons (PAHs) are known to be persistent, toxic, and linked to developmental abnormalities and physiological disruptions in aquatic species (Bagby et al., 2017; DeLorenzo et al., 2017; Gan et al., 2021; Hou et al., 2019; Meador & Nahrgang, 2019; Truskewycz et al., 2019; Whitehead et al., 2013; Zerebecki et al., 2022). Since the *Deepwater Horizon* oil spill in 2010, which released 4.6 million barrels of Louisiana sweet crude oil (LSC) into the Gulf Coasts, research on oil spill effects in salt marshes has grown. However, despite the increasing demand and transportation of petroleum products over the past 150 years, the majority of restoration methods have remained unchanged, and there are persisting data gaps in understanding the influence of bioremediation on these impacted areas (Demirbas & Taylan, 2016; Houghton, 1996; Kumar & Kaur, 2018).

Mycoremediation is a form of bioremediation where fungi are used to detoxify environmental pollutants, offering a natural and sustainable approach to mitigating their impacts (Akhtar & Mannan, 2020). Many oil-degrading fungi have been isolated from areas impacted by oil spills, and some species have been found to metabolize oil components more effectively than bacteria (Gan et al., 2021). This is due to the release of digestive enzymes that oxidize chemical bonds, including the ringed structures of many PAHs, leading to their mineralization (Kirchman, 2012; Zhuo & Fan, 2021). Additionally, the reduction of ringed structures increases the efficiency of microbial degradation processes (Acevedo-Sandoval et al., 2018). Research has also shown that *Pleurotus ostreatus*, commonly known as oyster mushrooms, alters the oil composition in polluted soils (Bhatt et al., 2002; White et al., 2006). A field trial reported that *P. ostreatus* mycelium produced fruiting bodies after inoculation in oil-contaminated soil piles (1%–2% = 10,000–20,000 ppm). In this

**Received:** March 28, 2024. **Revised:** October 01, 2024. **Accepted:** November 2, 2024

study, the oiled soil pile was reduced in 16 weeks to less than 200 ppm of oil, allowing plants, insects, and invertebrates to inhabit it. In contrast, control piles remained toxic to plants and worms (Thomas et al., 1999). This terrestrial research on soils is promising, but little research has been published evaluating mycoremediation in marine or estuarine systems (Šašek, 2003). Current media reports reference field trials using biodegradable floating hemp bags inoculated with mycelium on straw called a MycoBoom, a device that absorbs oil and acts as a corral to contain oil slicks. However, there are currently no reported data on the effectiveness of these booms in degrading oil on a large scale. With more research and understanding of these fungi as tools for bioremediation, compared to using heavy equipment to clean up oil, the in situ application of oil-degrading fungi may serve as a lower-cost and less invasive option (Akpasi et al., 2023). Also, these tools have the potential to engage local communities and serve as educational and collaborative initiatives to promote environmental awareness and involvement (Thomas et al., 2009).

Oyster mushrooms are promising candidates for the mycoremediation of oil spills in estuarine ecosystems. Their life cycle starts with the germination of spores that produce a branched network of fungal cells, or hyphae. These highly branched structures meet and combine to produce mycelium, which will grow along substrates and attach to their food source. The mycelium secretes digestive enzymes that break down large molecules into usable resources. They can be classified as "white rot" fungi, meaning they are primary degraders of lignin in woody substrates, resulting in the adaptation to metabolize complex hydrocarbons, including those in crude oil (Glumoff et al., 1990; Kirchman, 2012; Langer et al., 2021). They are also known for their low production cost, high yield, and high bio-adsorption potential due to their extensive biomass (Girmay et al., 2016; Kapahi & Sachdeva, 2017). Moreover, *P. ostreatus* is relatively simple to cultivate because it can thrive in diverse environments, including saline habitats (Bumpus et al., 1985; Da Luz et al., 2013; Rodrigues da Luz et al., 2012). This study aimed to examine how *P. ostreatus* mycelium growth dynamics are influenced by salinity ranges common to estuarine environments. Whereas previous research has focused on enhancing oyster mushroom fruiting body growth for increasing production (Elkanah et al., 2022; Girmay et al., 2016; Muswati et al., 2021), this study introduces the framework of a novel remediation approach by considering the stoichiometric ratio of carbon (C), nitrogen (N), and phosphorus (P) in a substance or organism (C: N: P) for growth and development (Chen et al., 2020; Tang et al., 2018). By understanding the specific C: N: P ratio of a target organism, nutrient amendments can be tailored to provide a balanced and optimal nutrient supply, promoting a more robust product while mitigating nutrient imbalances that may limit biodegradation performance (Yang et al., 2019). This targeted nutrient amendment strategy aligns with sustainable practices by potentially reducing the risk of nutrient runoff into water bodies and ecological damage (Liu et al., 2021). In addition, we explored how *P. ostreatus* and nutrient amendments affect the composition of crude oil through saturate, aromatic, resins, and asphaltenes (SARA) analysis.

# Methods

## Cultivation of P. ostreatus

*Pleurotus ostreatus* was obtained in a sawdust fruiting block from Field and Forest (Peshtigo, WI). Culturing substrate consisted of potato dextrose agar (PDA) or oatmeal agar (OMA) plates prepared by mixing 400 ml distilled water with 8 g dextrose (Sigma-Aldrich), 6 g agar (Sigma-Aldrich), and either 1.6 g potato starch (Millipore-Sigma) for PDA or 32 g strained cooked oatmeal for OMA. The solution was heated to 100 °C, stirred on a hot plate with a stir bar for 10 mins, and then autoclaved to 120 °C for 30 mins. The agar was poured (30 ml) into 100 mm Petri plates; the plates were covered and allowed to cool and solidify overnight. Sterile plates were then stored at 4 °C. To culture *P. ostreatus*, mycelium was directly transferred from the fruiting block to sterile agar plates and labeled as P0 for initial inoculation to agar. After 2 days, this mycelium was transferred to a new sterile agar plate labeled P1 (Stamets, 1993). If P1 mycelium growth reached the plate margin, a subsequent transfer followed and was identified as P2. Mycelium transfer cohorts were thus tracked as P1, P2, P3, and so forth, where all experiments contained mycelium from P1 to P3 to maintain consistency. Oyster mushroom mycelium has been shown to grow between 55 °C and 29 °C per fruiting block instructions, but cultures in this study were incubated at 25 °C to simulate average temperatures of the Gulf Coast. Mycelium on agar was stored at 4 °C until used for experiments (Stamets, 2000).

## Effect of salinity on mycelium growth

To determine the salinity tolerance of *P. ostreatus*, we measured mycelium growth over time in vitro with varying salinity concentrations under sterile conditions. For this test, PDA was prepared and sterilized. Aliquots of agar were amended with Instant Ocean in 600 ml glass beakers to achieve salinities of 0, 5, 10, 15, 20, 25, 30, and 35 g/L (‰). The salinity agar treatments were then mixed with a stir bar, and 30 ml were poured into Petri dishes, stored at room temperature while cooling, and allowed to solidify for 18 hrs and then transferred to 4 °C until use. Three plates for each salinity were inoculated with a 10 mm mycelium plug in the center of each plate and incubated at 25 °C for 7 days. Digital images were acquired using a standard frame to control for focal distance. They were taken every 24 hrs to determine surface area growth via the open-source National Institute of Health's FIJI Software. All other testing in this work included agar without salt amendments (0‰ salinity) in accordance with normal PDA and OMA agar recipes (Stamets, 2000).

## Effect of nutrient amendments on mycelium growth

The average C: N: P ratio of *P. ostreatus* was calculated to be 44:5:1 from supplementary data reported by Zhang & Elser (2017). The chosen nutrient amendments for nitrogen and phosphorus amendments based on the *P. ostreatus* C: N: P ratio were ammonium chloride ($NH_4Cl$), urea ($CH_4N_2O$), and potassium phosphate ($KH2PO_4$), as well as the combinations of the N and P solutions ($NH_4Cl + KH_2PO_4$ and $CH_4N_2O + KH_2PO_4$; Table 1). Nutrient stock solutions were prepared with sterile filtered deionized water. Fresh and/or weathered crude oil serves as the amended carbon source in the system based on the percent of carbon in petroleum crude oil, which is approximately 85% (Hyne, 2001). Both weathered and fresh LSC was obtained from Louisiana State University. Fresh LSC oil was a blend of crude oil from several reservoirs in offshore Louisiana. The oil underwent evaporation, reducing its weight by 25%–30%. It was achieved by passing air through it to force the evaporation of the light hydrocarbons sourced from a nearby oil drilling platform (referred to as fresh oil). The same oil was also subjected to artificial weathering through UV radiation and motion to simulate wave action (weathered oil). Samples were stored in sealed amber vials at 4 °C. The concentrations for carbon, nitrogen, and phosphorus ratios were calculated based on the molecular weight of each compound. For each treatment,

**Table 1.** Nutrient amendment treatment parameters based on *Pleurotus ostreatus* C: N: P of 44:5:1 where nutrient ratio levels were derived using 0.1 ml crude oil (for carbon, fresh, or weathered oil) to 0.05 g/ml $NH_4Cl$, 0.03 g/ml $CH_4N_2O$, (for nitrogen), and/or 0.01 g/ml $KH_2PO_4$ (for potassium).

| Oil amendment | Nutrient amendments | | | | | |
|---|---|---|---|---|---|---|
| **No oil** | None | $NH_4Cl$ | $CH_4N_2O$ | $KH_2PO_4$ | $NH_4Cl+KH_2PO_4$ | $CH_4N_2O+KH_2PO_4$ |
| **Fresh oil** | None* | $NH_4Cl$ | $CH_4N_2O$ | $KH_2PO_4$ | $NH_4Cl+KH_2PO_4$* | $CH_4N_2O+KH_2PO_4$* |
| **Weathered oil** | None* | $NH_4Cl$ | $CH_4N_2O$ | $KH_2PO_4$ | $NH_4Cl+KH_2PO_4$* | $CH_4N_2O+KH_2PO_4$* |

*Note.* The control group did not include oil. Sample groups also chosen for saturate, aromatic, resin, and asphaltene analysis were included in this table and indicated by (*).

they were used as follows: 0.1 ml crude oil (fresh or weathered) to 0.05 g/ml $NH_4Cl$, 0.03 g/ml $CH_4N_2O$, and/or 0.01 g/ml $KH_2PO_4$ (Table 1). Nutrient solutions were added to each PDA plate before inoculation of mycelium. Aliquots of 50 µl of each stock solution of either nitrogen, phosphorus, combinations of both nutrients, or deionized water (as a control) were added to the surfaces of Petri dishes. The dishes were then allowed to dry at room temperature for 24 hrs and labeled accordingly by nutrient. After 24 hrs, 0.1 ml of fresh or weathered oil was spread over the surface of the agar. Finally, 10 mm plugs of mycelium were extracted using a 10 mm core borer from the cultured community. The mycelium plugs were then placed on each PDA agar plate to assess the ideal mycelium growth at various C: N: P nutrient combinations. Inoculated plates were incubated at 25 °C, and images were taken every 24 hrs for 7 days to determine surface area growth via FIJI Software. Given a noticeable decrease in the pH of overlying water (~5.7 pH) in PDA in *P. ostreatus* mycelium and oil tests, oatmeal agar was used as the primary substrate for mycelium (Crescent et al. 2024, unpublished data) in all future testing.

## Effects of nutrient addition on oil composition

Agar dishes were prepared with OMA and *P. ostreatus* mycelium plugs with various oil and nutrient treatment factors (Table 2). To determine the possible effects of mycelium growth on changes in oil composition for carbon utilization, samples were collected from each treatment on Day 7 and Day 14 for SARA analysis. The SARA method used was adapted from methods detailed in Dettman et al. (2005), Dutta and Harayama (2000), and Kharrat et al. (2007). Agar samples were placed in 1,000 ml separatory funnels and soaked in 60 ml dichloromethane (DCM) for 18 hrs to separate hydrocarbons from the agar. The extracts were collected into tared 60 ml glass collection vials.

The sample extracts were concentrated to dryness using a nitrogen evaporator (Organomation, N-Evap 11) and weighed to determine the oil mass. Afterward, the dried oil samples were resuspended in 60 ml pentane, then sonicated for 45 mins, and allowed to sit for 18 hrs to enable the precipitation of the asphaltenes. The asphaltenes were collected by passing the pentane mixture through a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter (Thermo Scientific, 30 mm PTFE membrane) and rinsed with 60 ml of pentane. The deasphalted fraction (i.e., maltene fraction) was collected into a TurboVap tube (~120 ml total). The asphaltenes were then eluted from the filter using 50 ml of dichloromethane into a tared 60 ml collection vial. The DCM was removed via nitrogen evaporation and weighed to determine asphaltene mass. For the maltene fraction, samples were concentrated to 1 ml (Zymark TurboVap II concentration workstation, nitrogen 14 PSI water temperature = 40 °C), transferred into 4 ml amber autosampler vials, and brought up to 4 ml with pentane. Next, 1 ml of the maltene fraction (containing resins, aromatics, and saturates) was separated using 5 g silica solid-phase extraction columns. Saturates were first eluted with 50 ml hexane and then collected into a tared glass collection vial. Aromatics were eluted with 1:1 hexane/benzene (% v/v) and collected into a tared glass collection vial. Resins were collected into two tared collection vials, with 50 ml of DCM and 50 ml of methanol. Each sample was concentrated under nitrogen, and the mass of each fraction was measured and reported. The reported resin fraction is the sum of the DCM and methanol eluents. The proportion of each oil fraction was statistically analyzed using a two-way analysis of variance (ANOVA) with assigned factors of oil type (fresh or weathered oil), nutrient amendments (with or without nutrients), and time (7 or 14 days) transformed using arcsine square root transformation.

**Table 2.** The mean surface area ($mm^2$) of *Pleurotus ostreatus* mycelium at Day 7 of incubation with nutrient amendments in order from greatest growth to least growth.

| Treatment | Mean surface area ($mm^2$) | Standard deviation |
|---|---|---|
| $NH_4Cl + KH_2PO_4$ | 4012 | 534 |
| $NH_4Cl$ | 3249 | 393 |
| $KH_2PO_4$ | 2609 | 841 |
| $CH_4N_2O$ | 2165 | 189 |
| Control | 1911 | 416 |
| $CH_4N_2O + KH_2PO_4$ | 788 | 420 |

*Note.* Treatments include the no oil, fresh oil, and weathered oil treatments for each nutrient category.

## Data analysis

### *Imaging analysis*

The National Institutes of Health FIJI software was used to estimate the surface area of mycelium in each of the collected images (Figure 1). The experiment collected images in a darkroom using a 108 megapixel camera. Each imaging session used a calibration image to determine the global scale. Collected images were converted to a grayscale, and a highlight mask that included the mycelium was selected. The calibration image and the highlighted mask generated the surface area measure associated with inoculated mycelium. The surface area was recorded daily in Excel to evaluate the rates of mycelium growth over time.

### *Statistical analyses*

Subsequent surface area measurements determined calculated growth rates of mycelium for the various nutrient/oil treatments over time fitted to an exponential regression model in Excel. Growth rates were compared using ANOVA tests in JMP statistical software. Mycelium surface area data were analyzed using ANOVA and multiple comparisons of Tukey-Kramer tests. Saturate, aromatic, resin, and asphaltene values were compared via ANOVA tests using JMP statistical software.

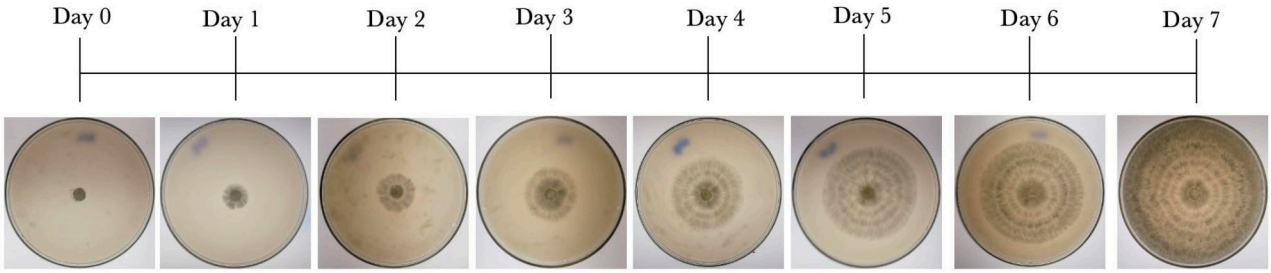


**Figure 1.** Potato dextrose or oatmeal agar in vitro where agar was amended with salts, nutrients, and oil as assigned before inoculation of freshly cut (10 mm) mycelium plugs. Plugs were placed in the center of the plates and incubated at 25 °C. Images were captured daily, and the surface area of mycelium was tracked using Image J software.

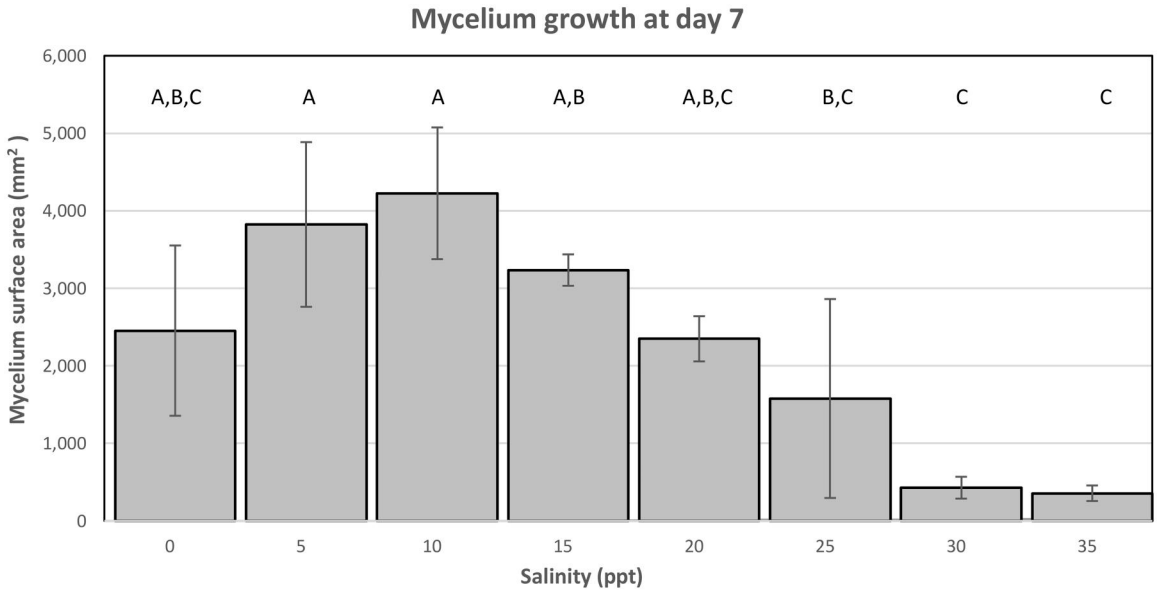


**Figure 2.** Surface area of *Pleurotus ostreatus* mycelium grown in vitro with varying salinities after 7 days at 25 °C. The range of salinities represents ranges typically found in coastal habitats. Salinities not connected by the same letter are significantly different, as determined by a one-way analysis of variance followed by a Tukey-Kramer test. Values represent the mean of replicates ± standard deviation.

# Results/discussion

## Mycelium growth in various salinities

Mycelium growth on PDA, reported as surface area, was recorded for 7 days over a range of salinities (0–35‰) typical of a southeastern U.S. estuary. Growth metrics (imaged surface area; Figure 2) indicate that *P. ostreatus* mycelium grew at all tested salinities, but growth was most robust between the 5–15 ppt range, simulating brackish or estuarine ranges in the natural environment (Figure 2). Comparisons of treatments for each pair using Tukey-Kramer's test resulted in significant differences between salinities in the 5–20‰ range (letter group A) and 0‰ including the 20–35‰ range (letter group C; Figure 2). Mycelium growth in this salinity range exceeded salinities >30‰ (Figure 2).

## Mycelium growth with nutrient amendments

To maximize *P. ostreatus* growth, optimal nutrient conditions must exist. Based on nutrient ratios found in *P. ostreatus* as reported by Zhang and Elser (2017), various nutrient (carbon as amended with LSC, nitrogen, and phosphorous ratio of 44:5:1) combinations were tested to evaluate the conditions that would maximize mycelium growth. Recorded surface area measurements were analyzed using a two-way ANOVA and multiple mean comparisons among oil type and nutrient amendments using JMP Ver. 12 software. Carbon sources for this test included fresh or weathered LSC and control PDA media (no oil; Figure 3). The results from Tukey-Kramer's test on the least square means indicate that the addition of oil had no significant impact on mycelium growth, and there was no significant difference between the oil types. These results suggest that carbon is unlimited within the system for mycelium to grow, regardless of adding oil. The two nitrogen sources for this test included urea ($CH_4N_2O$) or ammonium chloride ($NH_4Cl$), and the sole phosphorus source was potassium phosphate ($KH_2PO_4$). The two nutrient combinations included each nitrogen source with phosphorus ($CH_4N_2O$+$KH_2PO_4$) and ($NH_4Cl$+$KH_2PO_4$). The multiple comparison model comparing mean surface area results for nitrogen and phosphorus amended treatments indicated no significant differences between the control and single nutrient treatments $CH_4N_2O$ or $KH_2PO_4$ (Figure 3). The results show that nitrogen amendment $NH_4Cl$ had significantly increased mycelium growth compared to the control ($p = 0.0004$). This model indicated substantially higher surface area growth when mycelium was grown in the nitrogen $NH_4Cl$+$KH_2PO_4$ and a significant decrease in the recorded surface area of the $CH_4N_2O$+ $KH_2PO_4$ combination treatment. The multiple-factor analysis of variance indicated that oil type was not a significant factor ($p = 0.59$), but the nutrient amendments were the considerable drivers in mycelium surface area measurements ($p < 0.0001$). The interaction between oil type (carbon) and nitrogen or phosphorus (nutrient) treatments

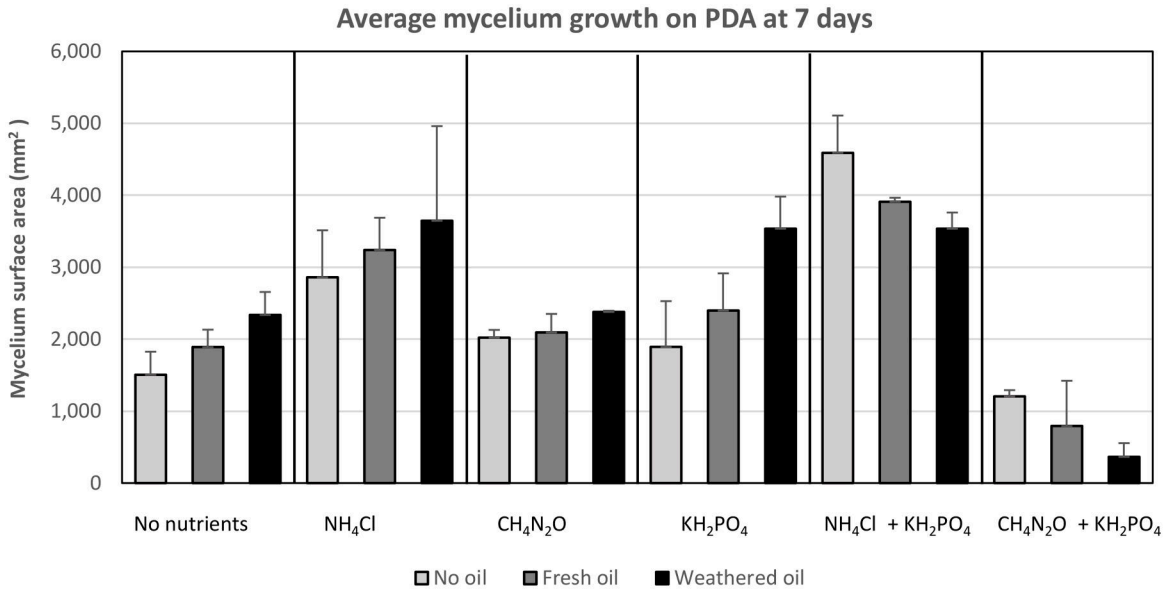


**Figure 3.** Average mycelium growth on potato dextrose agar (PDA) at Day 7, with the addition of Louisiana sweet crude and nutrient amendments concentrations based on the average C:N:P ratio (44:5:1) of *Pleurotus ostreatus*. Control treatment contained mycelium on agar. The following treatments included fresh or weathered oil and with or without nitrogen and/or phosphorus amendments.

did not significantly influence surface area growth ($p = 0.051$; Figure 3). The order of most significant growth enhancement to least was $NH_4Cl + KH_2PO_4$ > $NH_4Cl$ > $KH_2PO_4$ > $CH_4N_2O$ > Control > $CH_4N_2O$ + $KH_2PO_4$ (Table 2). These results align with previous research suggesting nitrogen is the primary nutrient limited during oil spill bioremediation, followed by phosphorus (Atlas & Hazen, 2011; Jabbar et al., 2022). Nitrogen is essential for synthesizing fungal cell walls, and phosphorus is vital for proteins, lipids, and nucleic acid synthesis (Megharaj et al., 2011). Oyster mushroom substrates typically include nitrogen and phosphorus-rich sources, such as wheat straw, spent coffee grounds, or cow manure. However, achieving a precise ratio of these nutrients can be challenging (Stamets, 2000). When selecting the nitrogen and phosphorus compounds for the in vitro experiments, we considered previous studies that looked at optimal growth conditions, which included ammonium chloride and potassium phosphate (Hoa & Wang, 2015). Notably, the combination of urea and phosphate significantly inhibited mycelium growth compared to the control (Figure 3). Previous research suggests urea can serve as a readily available nitrogen source for fungi when present in the environment but can be toxic to certain species (Navarathna et al. 2010; Veverka et al. 2007). Thus, it is essential to understand that the specific nutrient amendments required may vary depending on the organism, specific ecosystem, and the specific purpose for amending. Understanding the target organism's C: N: P ratio provides valuable information for developing effective nutrient-amending strategies that maximize benefits while minimizing potential drawbacks. To evaluate the efficiency of oil–mushroom–nutrient interactions, we must be able to track chemical changes in addition to mycelium growth rates in oil over time.

## Compositional analysis of oil with mycelium and nutrient amendments

Oil constituents change when exposed to surface conditions. Thus, it is crucial to identify and monitor changes in their composition (Overton et al., 2022). This study conducted a SARA analysis of the various oil–mycelium–nutrient treatments (Table 2). The saturate fractions were significantly affected by the presence of nutrients ($p = 0.0015$), time ($p < 0.0001$), and the interaction of nutrients*time ($p = 0.001$), but oil type did not significantly affect the saturate fraction. These results indicate that regardless of oil type, time and nutrients reduced the saturate fractions in fresh and weathered LSC (Figure 4). This supports previous studies showing that microbes' saturate oil fraction is more readily biodegradable (Gros et al., 2014; Wu et al., 2023). The multiple-factor analysis of variance indicated that the asphaltene fractions of LSC were significantly affected by nutrients ($p = 0.0124$), oil type ($p = 0.0189$), and the combined interaction treatment oil*time ($p = 0.0147$). These results indicate that *P. ostreatus* can alter the asphaltene fractions by adding nutrients in fresh oil (Figure 4). The aromatic fraction in fresh oil significantly differed from weathered oil ($p = 0.0178$). This is likely a volatilization factor (Bacosa et al., 2018) but may be influenced by bioremediation agents using available carbon for growth (Figure 4).

Additionally, the multiple-factor analysis test revealed that the proportion of resins in both oil types was significantly affected by time ($p = 0.0014$; Figure 4). Weathered LSC may contain higher proportions of asphaltenes, potentially influencing their alterations by the mycelium and nutrient combination. Asphaltenes are recalcitrant fractions in crude oil, and thus mycelium or other bioremediation agents could benefit from nutrient amendments to reduce (i.e., mineralize) these compounds. Saturates are the most prevalent component of LSC and are readily altered by various environmental factors and biological agents, including fungi, which was observed in this study. This fraction showed significant differences were reported for both time and nutrients ($p = 0.0010$). Resins generally may take longer to degrade as they aggregate in oil mixtures, limiting the fungi's ability to access them (Overton et al., 2022).

The surface area growth data of *P. ostreatus* in each treatment was recorded on Days 7 and 14, followed by oil extraction for SARA analysis to determine differences between adding fresh oil and the treatments amended with $NH_4CL+KH_2PO_4$. Image analysis data showed that after approximately 7 days, mycelium growth slows and reaches a stationary phase. This is often an indication of where growth becomes nutrient-limited. Still, in this

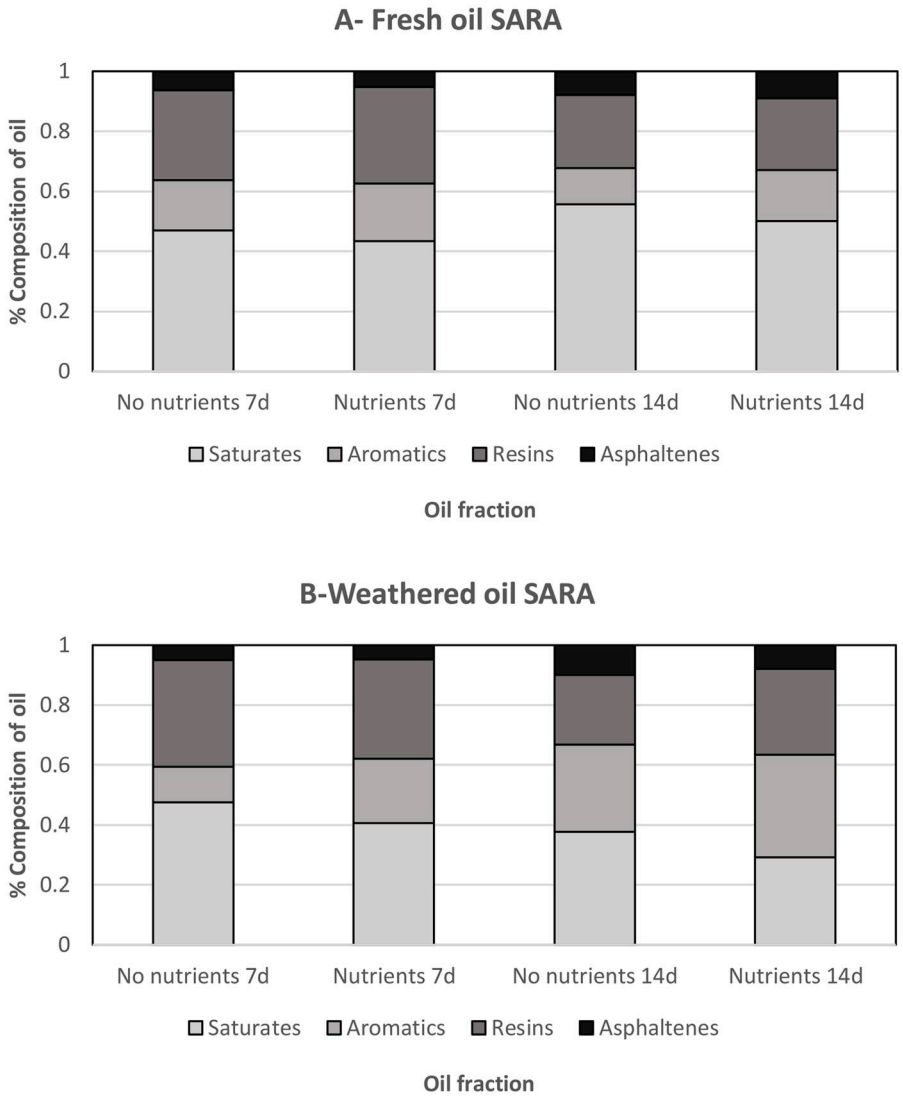


**Figure 4.** Percent composition of saturate, aromatic, resin, and asphaltene (SARA) components based on time and nutrients: (A) in fresh Louisiana sweet crude (LSC) oil and (B) in weathered LSC oil. The saturate fractions were significantly affected by time (analysis of variance [ANOVA], $p < 0.0001$) and nutrients (ANOVA, $p = 0.0015$). There was a significant decrease in aromatic fractions in fresh oil compared to weathered oil (ANOVA, $p = 0.015$). The proportion of resins were significantly affected by time (ANOVA, $p = 0.032$). The analysis revealed that the asphaltene fractions were significantly affected by nutrients (ANOVA, $p = 0.0124$), oil type (ANOVA, $p = 0.0189$), and the interaction of oil and time (ANOVA, $p = 0.0147$).

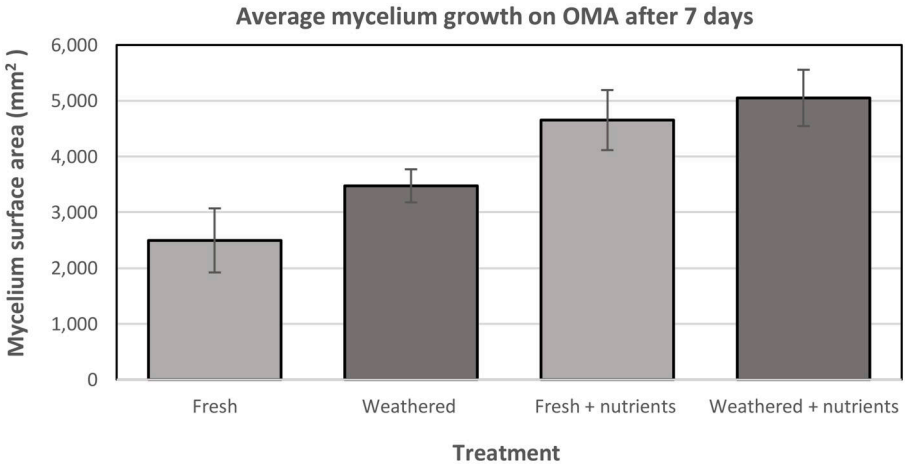


**Figure 5.** Average mycelium growth on oatmeal agar (OMA) at 7 days, with the addition of petroleum crude oil and nutrient amendments concentrations based on the average C:N:P ratio (44:5:1) of *Pleurotus ostreatus*. Control treatment contained mycelium on agar. The following treatments included fresh or weathered oil with or without either nutrient amendment. Results from all pairwise *t* test indicated significant differences ($p = 0.016$).

case, mycelium covered the entire surface area of the Petri dish and was limited by a physical boundary. Although the data also shows that by Day 7, treatments without oil had higher surface areas, the treatments containing oil and nutrients were markedly higher than oiled treatments without nutrients (Figure 5). A multifactorial repeated-measures ANOVA was performed on the mycelium surface areas recorded on Day 7. These results suggest that nutrients significantly affected the mycelium surface area ($p = 0.0164$). The Student $t$ test indicated that treatments with nutrients significantly differ from treatments without nutrients ($p = 0.016$). Furthermore, mycelium surface area measurements were significantly different in treatments, including fresh, weathered, and oil-free. They also suggest that surface area under treatments without oil (control) and weathered oil with nutrient amendments were significantly higher than treatments without nutrient amendments and fresh oil with nutrients.

Knowing that mycelium growth was affected by nutrient amendments and oil, it was important to compare mycelium growth rates within each treatment. The surface area data set (between Day 3 and Day 7) was parsed from the entire surface area data set, and the resulting linear trendline was determined to identify each replicate's growth rate (i.e., slope). The calculated growth rates were sequentially analyzed by two-way ANOVA, with assigned oil type and nutrient amendment factors. The interaction term of nutrients*fresh oil showed significantly increased growth rates ($p = 0.015$) between Days 3 and 7 compared to treatments without nutrients and the interaction of nutrients with weathered oil. These results indicate that when fresh oil is combined with nutrient amendments, a faster growth rate of mycelium occurs. Growth rates from Days 3–7 were then compared to the individual SARA proportions at Day 7 by one-way ANOVA. The results indicated that the saturate proportions decreased across all treatments as the growth rates and resin proportions increased. These results suggest that the oil proportions of saturates and resins relate to growth rate. However, future studies must confirm the relationship between LSC oil fractions and *P. ostreatus* mycelium growth rates.

Determining the mass fractions of oil associated with SARA analysis in fresh and weathered oil helped evaluate the possibility of mycelium-derived mycoremediation. The mass of total recovered oil in fresh oil was greater in mycelium treatments, and, inversely, the weathered oil total recovered mass was significantly greater in the controls (Table 3). The mass increase in fresh oil fractions may be impacted by sampling techniques, but it is well known that initial weathering of crude oil is being driven by well-documented abiotic processes such as evaporation (Lee et al., 2023; Lundstedt et al., 2007; Overton et al., 2022). Weathered oil tends to have more complex, recalcitrant hydrocarbons (like resins and asphaltenes), which are harder to degrade by natural processes. In this case, mycelium may provide unique enzymatic pathways that enhance the breakdown of these more resistant oil fractions. Our data show significant reductions in weathered oil saturate, aromatic and asphaltene fractions when mycelium is present, and these data (Table 3) are supportive of mycoremediation. In fact, results for each of these fractions indicated an approximate two-fold decrease when mycelium and weathered oil were incubated. Fresh oil degradation is more naturally driven by environmental processes, with mycelium playing a less effective or even inhibiting role in 14 days. Weathered oil degradation, on the other hand, benefits from the specialized enzymatic activity of mycelium, which is likely more effective in breaking down the complex hydrocarbons that persist in weathered oil. This difference highlights how bioremediation strategies may need to be targeted to the age of the oil spill (fresh vs. weathered). In this study, mycelium was found to be more effective in altering oil composition in older, weathered oils.

## Conclusion

This study describes the concept and potential for mycoremediation of oil in estuarine systems using *P. ostreatus*. The salinity tolerance of *P. ostreatus* mycelium was between 5 and 15‰ and declined markedly at salinities greater than 25‰. This work also examined the growth response of *P. ostreatus mycelium* to nutrient variations. Altering nutrient availability significantly impacted mycelium growth, as expected. However, identifying specific nutrient combinations (such as ammonium chloride and potassium phosphate) that will optimize mycelium growth is essential when assessing its mycoremediation potential. The nutrient assays emphasized the importance of tailored nutrient amendments, as specific nitrogen sources, like urea, can inhibit mycelium growth. This study aimed to define relationships between surface area, nutrient amendments, and oil fractions. The results indicate that nutrient amendments and time influence the proportions of SARA fractions in the oil, particularly affecting saturates and asphaltene fractions. The aromatic fraction displays differences between fresh and weathered oil, suggesting potential bioremediation effects. The findings indicated that mycelium and the N: P nutrient combination could alter recalcitrant fractions like asphaltenes, promising for enhancing bioremediation effectiveness. And, finally, the significant decreases in saturates, aromatics, and asphaltenes in weathered oil with the presence of mycelium support the presence of mycoremediation in this effort. Further research aimed at understanding the intricate relationships between oil composition, mycelium growth, and nutrient amendments is crucial for developing efficient mycoremediation strategies. Although mycoremediation has shown

**Table 3.** The average mass (and range) estimates of saturate, aromatic, resin, and asphaltene fractions from fresh and weathered oil at 14 days when incubated in agar plates with and without *Pleurotus ostreatus* mycelium.

| | Fresh oil | | | Weathered oil | | |
|---|---|---|---|---|---|---|
| **Oil fraction** | **No mycelium (g)** | **Mycelium (g)** | ***p*-value** | **No mycelium (g)** | **Mycelium (g)** | ***p*-value** |
| **Saturates** | 0.0220 (0.0015–0.0399) | 0.0305 (0.0259–0.0376) | 0.0207* | 0.0649 (0.0360–0.1058) | 0.0155 (0.0086–0.0290) | <0.0001* |
| **Aromatics** | 0.0067 (0.0046–0.0109) | 0.0071 (0.0035–0.0092) | 0.0309* | 0.0184 (0.0064–0.0285) | 0.0106 (0.0051–0.0201) | 0.0001* |
| **Resins** | 0.0065 (0.0047–0.0088) | 0.0093 (0.0041–0.0131) | 0.0399* | 0.0090 (0.0075–0.0113) | 0.0070 (0.0049–0.0127) | 0.7224 |
| **Asphaltenes** | 0.0013 (0.0005–0.0028) | 0.0024 (0.0016–0.0028) | 0.0074* | 0.0047 (0.0025–0.0073) | 0.0021 (0.0012–0.0028) | 0.0001* |

*Note.* Significant differences between mycelium and no-mycelium exposures are indicated by (*).

promise in laboratory and small-scale studies, large-scale field studies and real-world applications still need to be augmented to evaluate effectiveness and scalability in various environments and contamination scenarios. The regulatory acceptance and standardization of mycoremediation as a recognized remediation technique still needs to be reformed, calling for further research to develop guidelines, regulations, and protocols for implementing mycoremediation projects and addressing potential liability concerns. With this added knowledge, mycoremediation using *P. ostreatus* could offer a promising and environmentally friendly approach for mitigating oil pollution in estuarine ecosystems.

## Acknowledgments

To E. Overton for the Louisiana Sweet Crude oil donation.

## Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

## Data availability

The data that support the findings of this study are available on request from the corresponding author, Ed Wirth. The data are not publicly available due to privacy or ethical restrictions.

## Author contributions

Summer Crescent (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization), Emily Pisarski (Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation), Ed Wirth (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation), and Richard Long (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision)

## Funding

This presentation was made possible by the National Oceanic and Atmospheric Administration (NOAA) Office of Education, Educational Partnership Program with Minority-Serving Institutions awards #NA16SEC4810009, #NA21SEC4810004, and #NA16SEC4810000 (NOAA Center for Coastal and Marine Ecosystems I and II, and NOAA EPP/MSI Graduate Fellow). The contents of this presentation are solely the responsibility of the award recipient and do not necessarily represent the official views of the U.S. Department of Commerce, NOAA. Any opinions, findings, conclusions, or recommendations expressed in this report are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Commerce, NOAA.

## Conflicts of interest

The authors have no conflict of interests related to this publication.

## Disclaimer

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## References

Acevedo-Sandoval, O., Gutiérrez-Alcantara, E. J., Perez-Balan, R., Rodríguez-Vázquez, G., Zamorategui-Molina, A., & Tirado-Torres, D. (2018). Degradation of polycyclic aromatic hydrocarbons using bacterial isolate from the contaminated soil and white rot fungus *Pleurotus ostreatus*. *Applied Ecology and Environmental Research*, *16*, 3815–3829. https://doi.org/10.15666/aeer/1604_38153829

Akhtar, N., & Mannan, M. A. (2020). Mycoremediation: Expunging environmental pollutants. *Biotechnology Reports*, *26*, e00452. https://doi.org/10.1016/j.btre.2020.e00452

Akpasi, S. O., Anekwe, I. M. S., Tetteh, E. K., Amune, U. O., Shoyiga, H. O., Mahlangu, T. P., & Kiambi, S. L. (2023). Mycoremediation as a potentially promising technology: Current status and prospects—A review. *Applied Sciences*, *13*, 4978.https://doi.org/10.3390/app13084978

Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environmental Science & Technology*, 45, 6709–6715. https://doi.org/10.1021/es2013227

Bacosa, H. P., Erdner, D. L., Rosenheim, B. E., Shetty, P., Seitz, K. W., Baker, B. J., & Liu, Z. (2018). Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil. *The ISME Journal*, *12*, 2532–2543. https://doi.org/10.1038/s41396-018-0190-1

Bagby, S. C., Reddy, C. M., Aeppli, C., Fisher, G. B., & Valentine, D. L. (2017). Persistence and biodegradation of oil at the ocean floor following Deepwater Horizon. *Proceedings of the National Academy of Sciences*, *114*. E9–E16. https://doi.org/10.1073/pnas.1610110114

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*, 169–193. https://doi.org/10.1890/10-1510.1

Bhatt, M., Cajthmal, T., & Sasek, V. (2002). Mycoremediation of PAH-contaminated soil. *Folia Microbiological*, *47*, 255–258. http://dx.doi.org/10.1007/BF02817647

Bumpus, J. A., Tien, M., Wright, D., & Aust, S. D. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. *Science*, *228*, 1434–1436. https://doi.org/10.1126/science.3925550

Chen, Z., An, C., Boufadel, M., Owens, E., Chen, Z., Lee, K., Cao, Y., & Cai, M. (2020). Use of surface-washing agents for the treatment of oiled shorelines: Research advancements, technical applications, and future challenges. *Chemical Engineering Journal*, *391*, 123565. https://doi.org/10.1016/j.cej.2019.123565

Da Luz, J. M. R., Paes, S. A., Nunes, M. D., Da Silva, M. dC. S., & Kasuya, M. C. M. (2013). Degradation of oxo-biodegradable plastic by Pleurotus ostreatus. *PloS One*, *8*, e69386. https://doi.org/10.1371/journal.pone.0069386

DeLorenzo, M. E., Evans, B. N., Chung, K. W., Key, P. B., & Fulton, M. H. (2017). Effects of salinity on oil dispersant toxicity in the eastern mud snail, *Ilyanassa obsoleta*. *Environmental Science and Pollution Research International*, *24*, 21476–21483. https://doi.org/10.1007/s11356-017-9784-9

Demirbas, A., & Taylan, O. (2016). Removing of resins from crude oils. *Petroleum Science and Technology*, 34, 771–777. https://doi.org/10.1080/10916466.2016.1163397

Dettman, H., Inman, A., Salmon, S., Scott, K., & Fuhr, B. (2005). Chemical characterization of GPC fractions of Athabasca bitumen asphaltenes isolated before and after thermal treatment. *Energy & Fuels*, 19, 1399–1404. https://doi.org/10.1021/ef0497356

Dutta, T. K., & Harayama, S. (2000). Fate of crude oil by the combination of photooxidation and biodegradation. *Environmental Science & Technology*, 34, 1500–1505. https://doi.org/10.1021/es991063o

Elkanah, F. A., Oke, M. A., & Adebayo, E. A. (2022). Substrate composition effect on the nutritional quality of *Pleurotus ostreatus* (MK751847) fruiting body. *Heliyon*, 8, e11841. https://doi.org/10.1016/j.heliyon.2022.e11841

Gan, N., Martin, L., & Xu, W. (2021). Impact of polycyclic aromatic hydrocarbon accumulation on oyster health. *Frontiers in Physiology*, 12, 734463. https://doi.org/10.3389/fphys.2021.734463

Girmay, Z., Gorems, W., Birhanu, G., & Zewdie, S. (2016). Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express*, 6, 87. https://doi.org/10.1186/s13568-016-0265-1

Glumoff, T., Harvey, P. J., Molinari, S., Goble, M., Frank, G., Palmer, J. M., Smit, J. D. G., & Leisola, M. S. A. (1990). Lignin peroxidase from Phanerochaete-chrysosporium: Molecular and kinetic characterization of isozymes. *European Journal of Biochemistry*, 187, 515–520. https://doi.org/10.1111/j.1432-1033.1990.tb15333.x

Gros, J., Reddy, C. M., Aeppli, C., Nelson, R. K., Carmichael, C. A., & Arey, J. S. (2014). Resolving biodegradation patterns of persistent saturated hydrocarbons in weathered oil samples from the deepwater horizon disaster. *Environmental Science & Technology*, 48, 1628–1637. https://doi.org/10.1021/es4042836

Hoa, H. T., & Wang, C.-L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus and Pleurotus cystidiosus*). *Mycobiology*, 43, 14–23. https://doi.org/10.5941/MYCO.2015.43.1.14

Hou, Y., Li, Y., Liu, Y., Li, G., & Zhang, Z. (2019). Effects of polycyclic aromatic hydrocarbons on the UV-induced fluorescence spectra of crude oil films on the sea surface. *Marine Pollution Bulletin*, 146, 977–984. https://doi.org/10.1016/j.marpolbul.2019.07.058

Houghton, E. (1996). Climate change 1995: The science of climate change: Contribution of working group I to the second sssessment report of the intergovernmental panel on climate change. (Vol. 2). Cambridge: Cambridge University Press.

Hyne, N. J. (2001). *Nontechnical guide to petroleum geology, exploration, drilling, and production* (2nd ed.). Penn Well Corp.

Jabbar, N. M., Alardhi, S. M., Mohammed, A. K., Salih, I. K., & Albayati, T. M. (2022). Challenges in the implementation of bioremediation processes in petroleum-contaminated soils: A review. *Environmental Nanotechnology, Monitoring & Management*, 18, 100694. https://doi.org/10.1016/j.enmm.2022.100694

Kapahi, M., & Sachdeva, S. (2017). Mycoremediation potential of pleurotus species for heavy metals: A review. *Bioresources and Bioprocessing*, 4. https://doi.org/10.1007/978-981-13-1840-5_20

Kharrat, A. M., Zacharia, J., Cherian, V. J., & Anyatonwu, A. (2007). Issues with comparing SARA methodologies. *Energy & Fuels*, 21, 3618–3621. https://doi.org/10.1021/ef700393a

Kirchman, D. L. (2012). *Processes in microbial ecology*. Oxford University Press.

Kumar, R., & Kaur, A. (2018). Oil Spill Removal by Mycoremediation. In V. Kumar, M. Kumar, R. Prasad (Eds.), Microbial action on hydrocarbons. Springer, Singapore. https://doi.org/10.1007/978-981-13-1840-5_20

Langer, G. J., Bußkamp, J., Terhonen, E., & Blumenstein, K. (2021). Fungi inhabiting woody tree tissues. In F. O. Asiegbu, & A. Kovalchuk (Eds.), *Forest Microbiology* (pp. 175–205). Elsevier. https://doi.org/10.1016/B978-0-12-822542-4.00012-7

Lee, J., Piao, L., & Park, H. (2023). Characterization of the physical and weathering properties of low sulfur fuel oil (LSFO) and its spreading on water surface. *Journal of Hazardous Materials*, 453, 131444. https://doi.org/10.1016/j.jhazmat.2023.131444

Liu, L., Zheng, X., Wei, X., Kai, Z., & Xu, Y. (2021). Excessive application of chemical fertilizer and organophosphorus pesticides induced total phosphorus loss from planting causing surface water eutrophication. *Scientific Reports*, 11, 23015. https://doi.org/10.1038/s41598-021-02521-7

Lundstedt, S., White, P. A., Lemieux, C. L., Lynes, K. D., Lambert, I. B., Öberg, L., Haglund, P., & Tysklind, M. (2007). Sources, fate, and toxic hazards of oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites. *Ambio*, 36, 475–485. https://doi.org/10.1579/0044-7447(2007)36[475:SFATHO]2.0.CO;2

Meador, J. P., & Nahrgang, J. (2019). Characterizing crude oil toxicity to early-life stage fish based on a complex mixture: Are we making unsupported assumptions? *Environmental Science & Technology*, 53, 11080–11092. https://doi.org/10.1021/acs.est.9b02889

Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., & Naidu, R. (2011). Bioremediation approaches for organic pollutants: A critical perspective. *Environment International*, 37, 1362–1375. https://doi.org/10.1016/j.envint.2011.06.003

Muswati, C., Simango, K., Tapfumaneyi, L., Mutetwa, M., & Ngezimana, W. (2021). The effects of different substrate combinations on growth and yield of oyster mushroom (*Pleurotus ostreatus*). *International Journal of Agronomy*, 2021, 1–10. https://doi.org/10.1155/2021/9962285

Navarathna, D. H. M. L. P., Harris, S. D., Roberts, D. D., & Nickerson, K. W. (2010). Evolutionary aspects of urea utilization by fungi. *FEMS Yeast Research*, 10, 209–213. https://doi.org/10.1111/j.1567-1364.2009.00602.x

Overton, E. B., Adhikari, P. L., Radović, J. R., & Passow, U. (2022). Fates of petroleum during the deepwater horizon oil spill: A chemistry perspective. *Frontiers in Marine Science*, 9, 928576. https://doi.org/10.3389/fmars.2022.928576

Šašek, V. (2003). "Why mycoremediations have not yet come into practice" The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions (pp. 247–266). Kluwer Academic Publishers, Netherlands.

Rodrigues da Luz, J. M., Nunes, M. D., Paes, S. A., Torres, D. P., de, C., Soares da Silva, M., & Kasuya, M. C. M. (2012). Lignocellulolytic enzyme production of *Pleurotus ostreatus* growth in agro-industrial wastes. *Brazilian Journal of Microbiology*, 43, 1508–1515. https://doi.org/10.1590/S1517-838220120004000035

Stamets, P. (1993). *Growing gourmet and medicinal mushrooms: A companion guide to the mushroom cultivator*. Ten Speed Press.

Stamets, P. (2000). Growing gourmet and medicinal mushrooms (pp. 21–33). Ten Speed Press.

Stamets, P. (2005). *Mycelium running: How mushrooms can help save the world*. Ten Speed Press.

Tang, Z., Xu, W., Zhou, G., Bai, Y., Li, J., Tang, X., Chen, D., Liu, Q., Ma, W., Xiong, G., He, H., He, N., Guo, Y., Guo, Q., Zhu, J., Han, W., Hu, H., Fang, J., & Xie, Z. (2018). Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 4033–4038. https://doi.org/10.1073/pnas.1700295114

Thomas, S. A., Becker, P., Pinza, M. R., Word, J. Q. (1999). Mycoremediation of aged petroleum hydrocarbon contaminants in soil. *Washington State Department of Transportation*, 13–17.

Thomas, S. A., Aston, L. M., Woodruff, D., & Cullinan, V. (2009). Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the Dungeness Watershed, Washington. Final Report.

Truskewycz, A., Gundry, T. D., Khudur, L. S., Kolobaric, A., Taha, M., Aburto-Medina, A., Ball, A. S., & Shahsavari, E. (2019). Petroleum hydrocarbon contamination in terrestrial ecosystems—Fate and microbial responses. *Molecules*, *24*, 3400. https://doi.org/10.3390/molecules24183400

Veverka, K., Štolcová, J., & Růžek, P. (2007). Sensitivity of fungi to urea, ammonium nitrate and their quimolar solution UAN. *Plant Protection Science*, *43*, 157–164. https://doi.org/10.17221/2239-PPS

White, P. M., Wolf, D. C., Thoma, G. J., & Reynolds, C. M. (2006). Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. *Water, Air, and Soil Pollution*, *169*, 207–220. https://doi.org/10.1007/s11270-006-2194-0

Whitehead, A. (2013). Interactions between oil-spill pollutants and natural stressors can compound ecotoxicological effects. *Integrative and Comparative Biology*, 53, 635–647. https://doi.org/10.1093/icb/ict080

Wu, B., Xiu, J., Yu, L., Huang, L., Yi, L., & Ma, Y. (2023). Degradation of crude oil in a co-culture system of *Bacillus subtilis* and *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, 14, 1132831. https://doi.org/10.3389/fmicb.2023.1132831

Yang, S.-X., Li, F.-M., Peng, X.-Z., Cao, J.-B., & Gao, Z.-X. (2019). [Effects of amendments with different C/N/P ratios on plant and soil properties of a Pb-Zn mine tailings]. *Huan Jing ke Xue= Huanjing Kexue*, 40, 4253–4261. https://doi.org/10.13227/j.hjkx.201812190

Zerebecki, R. A., Heck, K. L., & Valentine, J. F. (2022). Biodiversity influences the effects of oil disturbance on coastal ecosystems. *Ecology and Evolution*, 12, e8532. https://doi.org/10.1002/ece3.8532

Zhang, J., & Elser, J. J. (2017). Carbon:nitrogen:phosphorus stoichiometry in fungi: A meta-analysis. *Frontiers in Microbiology*, *8*, 1281. https://doi.org/10.3389/fmicb.2017.01281

Zhuo, R., & Fan, F. (2021). A comprehensive insight into the application of white rot fungi and their lignocellulolytic enzymes in the removal of organic pollutants. *The Science of the Total Environment*, *778*, 146132. https://doi.org/10.1016/j.scitotenv.2021.146132



Published by Oxford University Press on behalf of the Society of Environmental Toxicology and Chemistry 2025.

Environmental Toxicology and Chemistry, 2025, 00, 1–10
https://doi.org/10.1093/etojnl/vgae078
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