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Pleurotus spp. as Agents of Mycoremediation: A Review

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Abstract:

Elemental and molecular contaminants of anthropogenic origin represent an increasing threat to virtually all terrestrial and aquatic environments as well as their surrounding atmosphere. The decontamination and restoration of these environments pose a significant and expensive challenge. Although chemical treatment or physical removal of contaminated substrates often is the most direct response to eliminate contaminants, due to the complexity and sensitivity of many natural systems, technologies involving biological remediation can provide viable alternatives. While plant, bacterial, and fungal organisms have the abilities to accumulate or to metabolize toxic compounds, fungal organisms are uniquely suited to do so. Their fast growth and reproduction together with rapid genetic recombination allows fungi to evolve adaptations to specific conditions quickly, as do bacteria, as well as forming large, pervasive, mycelial networks that support nutrient utilization, in some aspects functionally similar to the roots of plants. This review is intended to provide an overview of the current stage of knowledge on the utilization and potential for bioremediation of a particularly promising genus of fungi: *Pleurotus*, a genus of about 200 species which occur in nearly all terrestrial environments in the northern hemisphere. From a sampling of 32 peer reviewed studies of pure research, information has been compiled regarding the adsorption and biotransformation of recalcitrant hydrocarbon compounds and the adsorption and hyper accumulation of heavy metal ions. Based on the information gathered in this review, Pleurotus spp. have the potential to perform mycoremediation in response to a variety of contamination scenarios both in soils and wastewaters.

Introduction:

Mycoremediation, the process of using fungal species to replenish and decontaminate ecological systems, has garnered increasing attention in recent years (Chun et al, 2019). In their ecological roles as decomposers, plant symbionts, and pathogens, fungi have evolved a plethora of biosynthetic and metabolic pathways to efficiently utilize their diverse diets.

Species of the genus *Pleurotus*, a group of typically wood-decomposing fungi, produce edible mushrooms that are widely used for human consumption. For this reason, its spawn, mycelium, and the byproducts of its production are readily available and the means of its cultivation are well known. *Pleurotus ostreatus* (Chun et al, 2019), *P. eryngii* (Bystrzejewska-Piotrowska et al, 2008a), *P. djamor* (Chang et al, 2021), *P. pulmonarius* (Benitez et al, 2021), *P. dryinus* (Ariste et al, 2020) and *P. cornucopiae* (Xu et al, 2021) are among the species that have been investigated for their potential role in mycoremediation approaches of mostly hydrocarbon and metal contaminations.

Pleurotus species have been found to be effective at degrading a diverse range of hydrocarbons (García-Delgado et al, 2015). The hydrocarbon contaminants on which it has been tested include petroleum products (Mohammadi-Sichani et al, 2019), byproducts of plastic production (Kaewlaoyoong et al, 2021), industrial cleaning agents (Mayans et al, 2021), pharmaceuticals (Migliore et al, 2021), UV blockers in sunscreen (Yang et al, 2021) and dyes (Ibrahim et al, 2018). The prospects of this application are particularly promising as these species have been found to convert many common toxic chemical compounds into inert forms ready to harmlessly re-enter the carbon cycle (Chun et al, 2019).

Another potential application for mycoremediation through *Pleurotus sp*. lies in its capacity to hyper-accumulate heavy metals (Bystrzejewska-Piotrowska et al, 2008b). Lead, mercury, cadmium, and cesium all represent toxicity and health risks in soils and waterways. *Pleurotus* species are known to accumulate these metals at many times the levels found in their substrates. This capacity represents both a hazard for the consumer as well as a tool for the remediator to extract and condense these elements from the environment. Contrary to hydrocarbons, heavy metals retain their toxic potential after being taken up by the fungus and the fruit bodies or mycelium must be physically removed for decontamination to be complete at a given site. Although the disposal of heavy metal laden mushroom bodies represents a further challenge, perhaps with greater adoption of mycoremediative practices, processes could be developed to contain, sequester and even recycle these compounds from the harvested mushroom tissue.

Contaminated soil and solid debris substrates are obvious potential targets of *Pleurotus* remediation because this is the natural habitat of the organism. Under proper conditions, mycelial networks can be very extensive, far reaching, and can penetrate polluted substrate (Chun et al, 2019). Unlike methods of decontamination involving chemical treatments or wholesale removal of the polluted material which degrade the existing microbial diversity and structure of soil, mycelial inoculation contributes to its health. Although the process may be slower, the extraction of heavy metals and the degradation of hydrocarbon-based pollutants can be accomplished without disturbing the soil structure and the habitat as a whole.

A very recent approach of *Pleurotus* based remediation is the treatment of contaminated water. Wastewater from specific industries as well as public water works could potentially benefit. Water filters inoculated with *Pleurotus* mycelium as well as filters treated with enzymes produced from *Pleurotus* species have been tested for this purpose (Ariste et al, 2020).

Remediation potential is not limited to living *Pleurotus* fungi. Spent mushroom substrate contains byproducts of cultivation that have been shown to filter heavy metals and degrade hydrocarbons through biosorption and biotransformation (Ariste et al, 2020).

Literature Review Methods:

In order to assess the types and frequency of remediation applications for which *Pleurotus* species have been considered, 32 papers were selected from peer reviewed journals. 7 additional papers demonstrating reviews of a similar ilk were selected to provide context, edification, and to generally further familiarity with the subject. The search term '*pleurotus* mycoremediation' was used in Web of Science on October 22nd, 2022 and the results were ordered according to the greatest number of citations. The same search term was used again on December 13th, 2022 and the results were sorted in order of greatest relevance. A few articles that either showed negative results for the application of *Pleurotus* in a specific remediation target or that described a topic outside of our focus were discarded.

Results:

Selected papers were published between 2002 and 2022 with the average date of publication being 2016. Half of the papers sampled were published in 2019 or later. Two authors, Chang and Bystrzejewska-Piotrowska, appear as first author twice each. *P. ostreatus* was by far the most studied species. As is indicated in Figure 1, it occurred as the subject of experimental study 3 times as often as the next most studied species, *P. eryngii.* As illustrated in Figure 2, 24 studies addressed hydrocarbon pollutions of various types as the target of remediation while 10 addressed heavy metals. Outside of these dominant categories, single studies concerned humic acid, *E. coli*, and temperature as a growth condition for remediation applications.

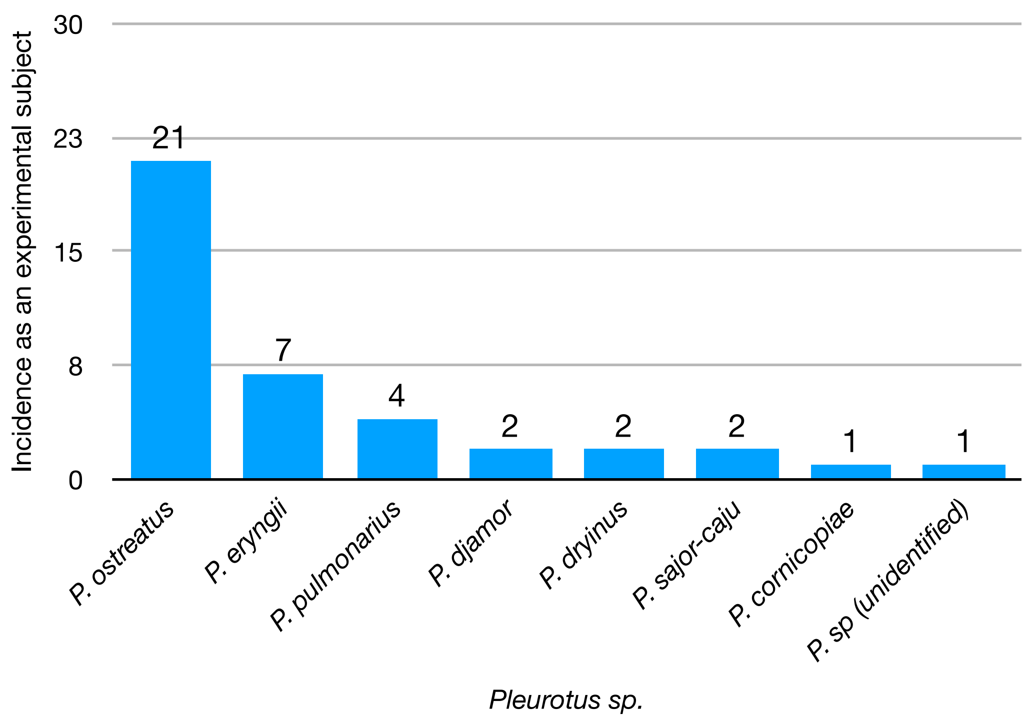

Fig. 1: Occurrence of *Pleurotus spp*. as the subject of studies within this review. Total incidence is greater than the total number of papers as some papers studied multiple species.

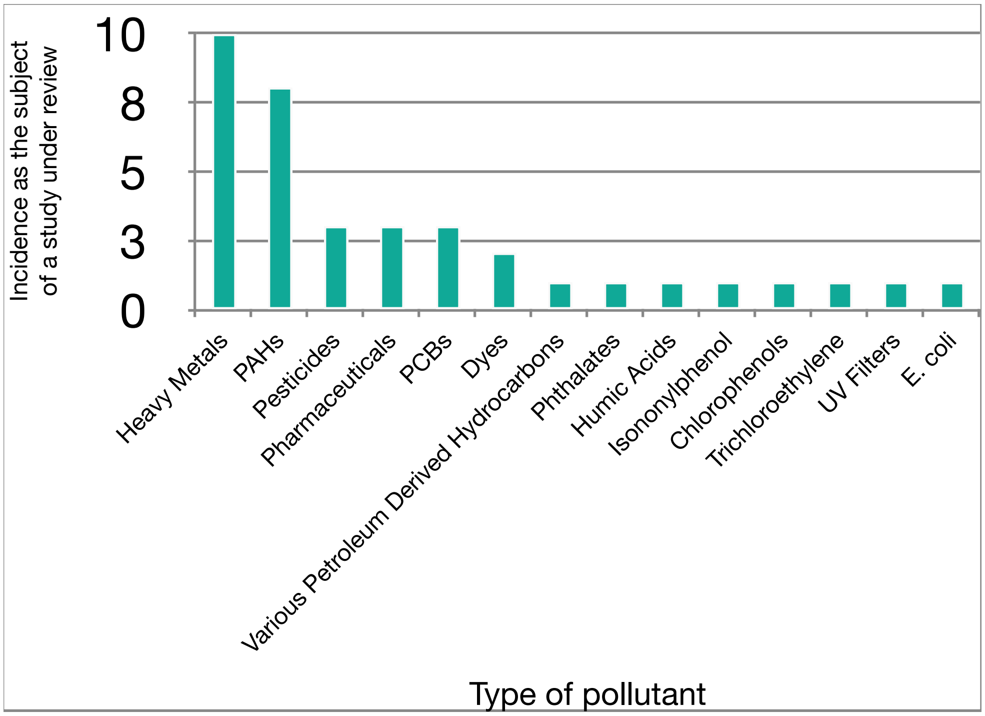

Fig. 2: Occurrence of types of pollutants as the subject of studies within this review. Each type of pollutant is considered a hydrocarbon with the exceptions of heavy metals, humic acids, and E. coli. Total incidence is greater than the total number of papers as some papers addressed pollutants from multiple categories.

Discussion:

Hydrocarbon Degradation:

Much of the designed chemical constituents intended for anthropocentric utility that ultimately escape into the environment fall into the broad designation of hydrocarbon pollution. *Pleurotus* in many instances have demonstrated the ability to degrade this type of molecule. With their near ubiquitous presence in the northern hemisphere, *Pleurotus* species are likely involved in myriad strategies to metabolize hydrocarbons wherever they encounter them in nature. Knowledge of the variability of degradation rates among different species and strains of *Pleurotus*, the impact of environmental conditions, efficacy of expedients, and the mechanisms of biochemical pathways involved in the breakdown of various hydrocarbons may prove helpful to improve remediation approaches.

A 2021 study published by Benitez et al. evaluated strains of *P. pulmonarius* and *Trametes sanguinea* with respect to their tolerance, removal capacity, toxicity reduction, and ligninolytic enzyme expression when cultivated in a technical mixture of polychlorinated biphenyls (PCBs) commonly used in transformer oil. *Pleurotus pulmonarius* out performed *T. sanguinea* removing 95% of the contaminants within a 24-day period.

Factors surrounding the degradation of PCB's by *P. sajor caju* were observed in Sadonoski et al (2019). These included removal capacity, toxicity reduction, morphological effects, and changes in laccase expression as well as a cost benefit analysis corresponding to the potential use of this strain for this remediation application. Removal of PCB's ranged from 91.7% to 97.7% depending on the substrate along with a concurrent toxicity reduction. Hyphal enlargement, change in total biomass, glucose metabolism, and protein expression were observed in the presence of PCBs and the Aroclor solution in which they were delivered. A threefold increase in laccase activity as well as a significant increase in laccase transcribing mRNA were observed.

The influence of heavy metal salts and ligninolytic enzyme mediators on the performance of *P. ostreatus* to degrade Benzo[a]pyrene, a poly-aromatic hydrocarbon (PAH) was investigated by Bhattacharya (2014). Metal ion salts and mediators both showed facilitatory contributions with copper ion salt outperforming manganese and zinc and the natural mediator vanillin outperforming synthetic forms of laccase mediator.

An investigation of the co-removal of manganese and the PAH phenanthrene by *P. eryngii* in the presence of the surfactants Tween 80 and saponin was conducted by Wu et al (2016). While Tween 80 aided fungal growth and saponin inhibited growth, both substances ultimately increased the remediating action of *P. eryngii* by increasing the solubility and bioavailability of the contaminants. A 93.85% degradation of phenanthrene after 15 days by *P. eryngii* supported by Tween 80 led the authors to conclude that co-treatment with Tween 80 is a promising myco-remediation strategy.

Moeder et al. (2005) analyzed the degradation efficiency and uptake of PCBs by *P. ostreatus* with respect to specific chemical structural aspects. The authors found certain chlorination grades as well as chlorine and carbon substitution patterns to be somewhat more resistant to degradation. Uptake of these more resistant constituents into the fruiting bodies of *P. ostreatus* was observed but only in trace, non-toxic amounts representing less than 0.1% of the total substrate contamination.

These studies demonstrate that the investigation of hydrocarbon degradation by Pleurotus has been vibrant in recent years. Efficacy rates between species, the influence of metal salt exposure, the potential of surfactants to increase pollutant bioavailability, post-degradation biomarker expression, and the susceptibility of particular hydrocarbon structural elements to degradation by *Pleurotus* species are among the subjects of contemporary research.

Heavy Metal Accumulation:

In addition to naturally occurring deposits of heavy metal in soils, industrial production often involves the creation and use of substances containing high concentrations of heavy metals. These include material products as well as by-products of material production and/or transportation. Notable sources of heavy metal pollution include the refining and burning of fossil fuels, mining projects, and the agricultural application of herbicides and insecticides. High concentrations of heavy metals are poisonous to many forms of life including microbiota, flora, and fauna, humans notwithstanding and are known to decrease population robustness and biodiversity within habitats. In some cases, *Pleurotus* species have been observed to hyper-accumulate heavy metals from the substrates they occupy. A detailed understanding of the optimal conditions, species capabilities, and uptake mechanisms may aid in the targeted remediation of acute contamination sites.

A study conducted by Bystrzejewska-Piotrowska et al (2008a), examined the influence of alkali and alkaline earth metal salts as well as tetraethylammonium salt on the uptake of metals by *P. eryngii*. Cesium, potassium, sodium, rubidium, and calcium salts were tested. The findings that Ca2+ did inhibit cesium uptake, tetraethyl ammonium had no influence, and K+ stimulated the uptake allowed the authors to infer that two pathways for cesium uptake were possible. One pathway utilizes a non-specific potassium channel on the plasmalemma, while another involves extracellular transport to the fruiting bodies by way of inter-hyphal cavities.

Bystrzejewska-Piotrowska et al. (2008b) further published the results of a similar study of earth metal uptake in *P. eryngii*. Here cesium, potassium, calcium, and sodium ions were evaluated with respect to accumulation and distribution within the stipe and cap as well as the relative effect their various concentrations bore on the uptake of the others. Transfer factors for each metal evaluated differed with cesium having the highest rate of transfer followed by potassium, sodium, and calcium. Higher concentrations of each ion tended to have a limiting effect on the uptake of the others. A control conducted without supplementation of potassium, sodium, or calcium resulted in a 62% uptake of the cesium present within the substrate into the fruiting bodies.

In 2018, Faria et al. tested various concentrations of the lithium compounds L Li_2CO_3 and LiCl in a liquid malt extract medium to determine the optimal conditions for bioaccumulation of the metal by *P. ostreatus*. Li2CO3 at a concentration of 40 mg/ L^{-1} showed the highest uptake rates.

Chen et al. (2018) looked at 43 strains of *P. ostreatus* collected from across China in order to identify strains with high tolerance for cadmium. Two strains demonstrated an exceptional ability with a maximum concentration tolerance of 80 mg/ L^{-1} , although peak accumulation occurred at 20 mg/ L^{-1}.

A genetic level analysis of *P. ostreatus* and *P. cornucopiae was* performed by Xu et al. (2021) to determine which genes were upregulated during cadmium exposure. Changes in enzyme production allowed for the inference that ATP cassette transport was utilized in *P. cornicopiae* while endocytosis and phagosome induction pathways were activated in *P. ostreatus* for cadmium removal from the substrate. A down regulation of glutathione metabolism and an upregulation of the mitogen activated protein kinase signaling pathway was observed in both species and assumed to be cadmium uptake resistance mechanisms by the authors.

This collection of recent studies concerning the metabolic pathways, interspecies and inter-strain capacity comparison, uptake distribution, and bioavailibity among compounds show active current research on *Pleurotus* species and their accumulation of heavy metals. Metals are not metabolized by *Pleurotus* (as hydrocarbons are) so their removal is likely more incidental than adaptive. Tolerance, however, is adaptive since tolerance provides a selective advantage at contaminated sites. A comprehensive catalog of *Pleurotus* species selected for their high tolerance and accumulation properties would be very useful for the targeted remediation of acutely polluted sites.

Implementation of *Pleurotus* production byproducts:

Typically, commercial production of edible mushrooms, including *Pleurotus* species, occurs on blocks of substrate made from wood chips or sawdust fixed together with a paste made from grain flours and an inoculum of the species intended for cultivation. Once manufactured and inoculated, these blocks go through an initial period of fruiting after which they are depleted and their ability to contribute more mushroom bodies declines over time. The spent mushroom compost (SMC) that results normally outweighs the amount of produced food by a factor of 5 (Chang et al, 2021). In addition to being used as an organic farm or garden compost that can further inoculate those soils, SMC contains large quantities of the enzymes that are the active agents of hydrocarbon degradation. This occurs because mycelia consume substrate through extracellular digestion. These digestive enzymes include but are not limited to certain laccases and peroxidases. In Chang et al. (2021) 72 distinct digestive enzymes were found in SMCs where just 3 species had been grown with 43 enzymes produced by *P. ostreatus* alone.

Because enzymes function outside of the living organism, they can be isolated or released from the SMC for remediation purposes. Organic pollutants shown to be degraded by living *Pleurotus* metabolism can potentially be degraded through associated digestive enzyme species alone. Indeed, Chang et al (2022) and Yang et al (2021) demonstrated degradation by extracted enzyme medium alone independent of living fungal organisms.

Along with digestive enzymes, *Pleurotus* have also been shown to produce certain bio-surfactants and emulsifying agents as a strategy to increase the bioavailability of hydrophobic hydrocarbon contaminants. In Pozdnyakova et al (2022) foams were produced by *Pleurotus sp.* at the interface between mycelia and contaminated substrate. Emulsifying agents were found to be highly dependent on contaminant exposure and varied greatly not only between species but between various strains within a given species. Indeed, *P. ostreatus* 'Florida' demonstrated a 70-fold increase in biosurfactant production in response to hydrophobic pollutant exposure while the 'MUT' *P. ostreatus* strain merely doubled its production rate. As is the case with enzymes, the presence of mycelial generated bio-surfactants is a considerable factor in the usefulness of SMC as well as a potential source of isolated medium of this type for the same purpose.

Mycelium fiber itself is another potentially useful byproduct of *Pleurotus* cultivation. Structural attributes of mycelium make it capable of the adsorption of heavy metals and some hydrocarbons. Along with enzymes, SMC contains large quantities of mycelium and can therefore be broken up or dissolved for adsorption. Mycelium can also be cultivated for easy extraction and integration into adsorptive filters for liquid medium decontamination.

Metabolic Pathways:

Many fungi, including all of those in Phylum Basidiomycota in which *Pleurotus sp.* belong, consist primarily of well-developed and complex fibrous networks of mycelial hyphae capable of weaving myriad paths through soils and the intercellular spaces of seemingly solid materials such as wood. Wood is composed of roughly 25% lignin, 45% cellulose, and 25% hemicellulose. Unlike so called ‘brown rot funguses’ that primarily consume the cellulosic carbohydrates and leave the ruddy oxidized lignin of wood behind, as white rot funguses, *Pleurotus sp*. readily consume the lignin as well. They do this initially through the extra-hyphal, extra-cellular release of digestive enzymes that work to chemically metabolize, mineralize, and generally disassemble these tissues into smaller components. These pieces can then be transported along the surface of the hyphae or absorbed into the hyphae where transportation and further break down may occur.

Because Pleurotus is capable of releasing a great variety of these enzymes, and especially because the most prominent of these are highly non-specific, they are incidentally capable of the deconstruction of many synthetic, otherwise highly persistent compounds. Enzyme non-specificity was highlighted in Kaewlaoyoong et al.’s (2021) study of *P. pulmonarius’s* degradation of PCDD/F in which 64 distinct derivative metabolites were identified compared to only 20 in the uninoculated control. Variety of metabolites relates directly to the enzymatic versatility as a greater number of points of biotransformation result in a higher number of bio-transformed constituents.

The two enzymes most often cited and studied in the papers within this review are laccase and manganese peroxidase. Both are ligninolytic. Laccase is especially notable for environmental consideration as it uses only molecular oxygen as a co-substrate and produces only water as a product, the material being bio-transformed notwithstanding. Manganese peroxidase production is believed to be unique to phylum Basidiomycota. Both Operate primarily through oxidation of aromatic rings within phenolic compounds.

Although biotransformation by *Pleurotus* can exhibit a high diversity of mechanisms, some patterns have been noted. Kozka et al. (2020) showed degradation of phamaceuticals that resemble monolignols through demethylation and oxygenation. Similarity between the target pollutant and components of the species natural substrate suggest remediative potential. Kaewlaoyoong et al. (2021) showed speed of degradation of PCDD/F positively correlated to the degree of molecular chlorination. Moeder et al. (2005) found similar results in their study of PCB mycoremediation including increased success when the chlorination occurred in the orthosubstituted ring position. In Grabarczyk et al.’s (2020) study *Pleurotus* strains were tested for their ability to degrade bicyclic halolactones. Hydrolytic dehalogenation, double bond epoxidation, and hydroxylation in the allyl position with possible further oxidaition to a carboxyl group were involved in the breakdown of the halolactone rings.

In a study by Chang et al. (2021) the degradation of phthalates is described by a two-step process by which esterases degrade phthalates to phthalic acids through hydrolysis in the first step, and aromatic ring cleavage occurs in the second step by decarboxylates, oxygenases, and oxidases/dehydrogenases. Roshandel et al. (2021) highlight increased tyrosinase activity when *P. florida (ostreatus)* degraded gas oil. It's mechanisms include the hydroxylation of monophenols to diphenols in a cresolase reaction and the oxidation of diphenols to quinones in a catecholase reaction. While the degradation strategies of Pleurotus enzymes are many, the primary means of biotransformation in many instances is laccase which uses copper to catalyze single electron reduction reactions.

In many of the studies within this review, enzymatic production was shown to be induced by exposure to the toxin being proposed for degradation. Kozka et al. (2020) showed *P. ostreatus* producing a 3 fold increase in laccase in the presence of the pharmaceutical contaminants it was shown to degrade. In Sadanoski et al. (2019), data was collected at the transcription level of this response. A 91% increase in laccase coding mRNA of *P. sajor-caju* exposed to polychlorinated biphenyls was measured. This mechanism does not always demonstrate contaminant-to-degrading-enzyme fidelity. Mayans et al. (2021) showed up-regulation of both MnP and laccase in both *P. ostreatus* and *P. eryngii* when exposed to trichloroethylene despite only the peroxidase showing involvement in degradation.

Bioavailability is an especially important factor in extra-organismal digestion because the conditions are only partially regulated by the fungus. Pozdnyakova et al. (2019) identified high solubility and low ionization potential as contributing factors to fungal availability. They were able to show congener specific degradation success by *P. ostreatus* occurred in the order anthracene < phenanthrene < fluorene, with fluorene representing the goldilocks condition and showing the greatest incidence of biotransformation, findings that agreed strongly with this theory. As described in the previous section, various biosurfacants and emulsifying agents are emitted from the hyphae with demonstrated dependency on species and the exogenous stimulant. In addition, Wu et al. (2016) showed correlation between increases in the enzyme production response and the presence and concentration of the synthetic biosurfacant Tween 80. This makes intuitive sense as increases in bioavailability effectively act as increases in exposure. Clearly there exists a concerted enzyme and surfactant response that includes elements of positive feedback.

Assuming bioavailability and extra-organismal digestion occur, uptake and transformation may follow. Bystrzejewska-Piotrowska et al. (2008b) suggest 4 zones of focus for assessing transport of ions; uptake into the interhyphal spaces, transport into the hyphae, movement along the hyphae, and movement within the fruiting body. Mechanisms associated with these actions include capillary forces, passive and active cellular transport, secondary transport, the influence of hyphal density, as well as the actions of the stipe cap interface and the fruiting body's elongation, differentiation, and biosynthesis. They note the role of potassium in enzyme regulation, homeostasis, and carbohydrate metabolism and calcium's role in the transport of monovalent ions. In a study conducted by Xu et al. (2021), differential stress responses to contaminant exposure among Pleurotus spp. were identified. ATP binding cassette transporters were more upregulated in *P. cornicopia* while the endocytosis and phagosome pathways were more upregulated in *P. ostreatus.* These responses were observed as reactions to high cadmium concentration stress and were identified as internal detoxification strategies of the fungus. In addition to ion diffusion and distribution, enzymatic digestion may continue inside the hyphae. P450 monoxygenase was identified by Kaewlaoyoong et al. (2021) as a particularly active hydrocarbon transformer that operates continued metabolization within the hyphae of Pleurotus spp.

A detailed understanding of the metabolic pathways associated with Pleurotus is essential to determining which species, if any, may be effective at removing a given contaminant. Taken together, the findings of the papers under review portray a nuanced illustration of these pathways and may yield insights into opportunities by which human efforts might support a desired decontamination objective.

Environmental Conditions:

There are many factors that can be taken into consideration regarding the conditions at the site of remediation. Whether the project concerns liquid medium or soil, knowledge and potential manipulation of these conditions can aid in the evaluation of whether mycoremediation is appropriate, help in the selection of the appropriate species and strains, and ultimately improve results.

The target pollutant may inhibit the growth or function of the fungus. In Pozdnyakova et al.'s (2022) study, the target pollutant isononylphenol inhibited the growth of *P. ostreatus*. This hazard may exhibit species specificity. Zahmatkesh et al. found exposure to the humic acid they were intent on removing from waste water had an inhibitory effect on *P. pulmonarius* and a slight excitatory effect on *P. ostreatus*. Although no studies in this review identified a degradation rate limited on one extreme by a low concentration and on the other by a high concentration, it should be assumed that such curves exist and maximum rates could be uncovered through further experimentation. By way of illustration, Njoku et al. (2018) found *P. pulmonarius*'s ability to degrade Dichlorvos pesticide directly related to the concentration in the soils it was exposed while Vaseem et al. (2017) found *P. ostreatus*'s ability to remove heavy metals from coal washery effluent directly related to the level of dilution. In one instance lowering the contaminant concentration lowered the rate and in the other lowering the contaminant concentration increased the rate. Effects may not be limited to fungal growth inhibition. Mayans et al. (2021) observed microscopic structural distortions at the hyphal level in *P. ostreatus* exposed to trichloroethylene. These changes were further accentuated with exposure to increased concentrations. No such morphological shifts were observed in *P. eryngii* under the same exposure. Although both species were able to complete 100% degradation of the trichloroethylene, the species that did not exhibit hyphal changes did so more quickly. Contaminant driven inhibition was the focus of a study by Chen et al. (2018) in which 43 strains collected from location across Northern China were compared on the basis of cadmium tolerance and 2 were identified as highly tolerant and adsorptive of the heavy metal ion.

Ion concentrations coincident with the target may exert influence as well. High concentrations of heavy metal ions showed inhibitory effects in several studies.

Bhattacharvya et al. (2014) found both growth and degradation ability of *P. ostreatus* to be severely limited by silver, cadmium, and mercury. Mohamadhasani et al. (2022) found similar results with high concentrations of copper and cobalt inhibiting hyphal development and severe intolerance to nickel even in low concentrations. The antioxidant enzymes catalase and superoxide dismutase increased dramatically in this study of *P. ostreatus* and were discussed by the authors as evidence of the fungal heavy metal stress response. Chang et al. (2021) found a decrease in phthalate degradation capability in the presence of magnesium, however, an increase was demonstrated in the presence of copper. The presence of copper supported hydrocarbon degradation in Bhattacharvya et al. (2014) as well. Copper and manganese both demonstrated monotonic effects on degradation potential with low levels supporting the mechanisms and high concentrations having an inhibitory effect. Notably these two ions are specifically those utilized by the most prominent catabolic enzymes laccase and manganese peroxidase as reducing agents. Generally, we can consider that high heavy metal concentrations have the potential to induce hazard through the heavy metal stress response but some access to trace levels of ionic copper and manganese may support the production of the enzymes necessary for hydrocarbon biotransformation.

Several other miscellaneous parameters were noted across this review including pH, soil organic matter (SOM) levels, biochar and vanillin amendment, nitrogen levels, and presumed support of bacteria populations in hydrocarbon biotransformation. Testing at pHs of 5, 7, and 9, Ibrahim et al. (2018) found pH 5 to be most favorable for the application of dye decolorization. Njoku et al. also found lower pH levels more conducive to the breakdown of dichlorvos pesticides. Indeed, Roshandel et al. showed laccase itself to achieve peak performance at pH 4.2. Njoku et al. (2018) as well as Mayans et al. (2021) showed SOM level as a potential limiting factor in pesticide and trichloroethylene respectively while Ranthankumar et al. (2020) identified a 9% SOM soil composition optimal for the decomposition of PAH's by P. dryinus. Garcia-Delgado et al. (2015) found biochar amendment increased the ability of *P. ostreatus* to decontaminate creosote compromised soils. Bhattacharvya et al. (2014) demonstrated vanillin amendment as a promoter of laccase-based PAH transformation noting its ability to supply the single enzymes necessary for the enzyme's reduction faculty. Zahmetkesh et al. (2017) found increased decolorization and conversion to folic acid like molecules of the waste water humic acids they targeted in nitrogen sufficient conditions. Sadanoski et al. (2019) observed similar results for *P. sajor-caju* conversion of PCBs. Mayans et al. (2021) discuss the positive influence of SOM in terms of its ability to harbor and support communities of potentially symbiotic bacteria.

In summary, when evaluating the potential for mycoremediation for a particular application, it can be important to consider the contaminant's effect on fungal growth and metabolisms. In addition, because co-contamination is more often the rule rather than the exception and because heavy metal distribution is pervasive due to both natural and human activities, it may be important to monitor these levels. While heavy metals may be a hazard, trace occurrence of specific elemental ions of copper and manganese may be necessary for fungal production of digestive enzymes. Low pH, biochar and vanillin amendment, ample nitrogen, and significant bacteria supporting

SOM all represent opportunities to assess or aid in the speed and efficiency of bioremediation by *Pleurotus spp*.

Waste Water Treatment:

11 of the 32 papers reviewed focused primarily on the decontamination of waste water by Pleurotus sp. Their focuses included applications to remove hydrocarbons and heavy metals, similar to many of the soil based investigations. The study conducted by Wu et al. (2016) investigated the simultaneous co-removal of the hydrocarbon phenanthrene and the heavy metal manganese with positive results.

Several of the contaminants targeted were of more specific concern to waste water treatment. These included the removal of widely used pharmaceuticals. Often un-metabolized portions of ingested drugs that are bio-reactive by design are excreted into the wastewater system and represent significant removal challenges. Chang et al. (2018) showed removal of sulfonamides and acetaminophen by *P. eryngii* while Kozka et al. (2020) demonstrated removal of anti-depressants and immunosuppressants by *P. ostreatus*. Other positive results were shown for the removal of UV blockers that enter waterways from the use of sunscreens (Yang et al., 2021), anthraquinone dyes released from fabrics (Pozdnyakova et al, 2022), and humic acids, a constituent often valued in soils but undesirable in water (Zahmatkesh et al., 2017). In a lone study of *Pleurotus sp*. as an antibiotic agent contributed by Pini et al. (2020), 99% removal of E. coli from both lab grown samples and contaminated samples from the Chicago river was performed by *P. ostreatus*. These results were even more remarkable in that the control containing no substrate (straw in this case), inoculated or otherwise, showed no change in *E. coli* population levels and the control containing un-inoculated substrate showed a 3-fold increase in *E. coli* population.

Because wastewater treatment typically involves the movement of relatively large amounts of the water across mycelial networks, the adsorptive faculty of the mycelium is considerably more important than the role of digestive enzymes and induction into the hyphal bodies. In fact, adsorption is often a precondition of the other mechanisms in liquid medium. Additionally, Chang et al. (2018) and Yang et al. (2021) observed higher rates of adsorption and biotransformation in slower flow rates. Similar to the monotonic relationship resulting from concentration based access and toxicity based inhibition, it should be considered that optimal flow rates for a given contaminant that balance exposure rate with the specific moment of adsorptive force exist and could be conclusively demonstrated with further experimentation.

Conclusion:

The purpose of this review was to elucidate a comprehensive understanding of current research concerning the potential utility of *Pleurotus* fungus for remediation applications. Evidence from 32 peer reviewed journals has described a genus capable of metabolizing a wide array of functionally dissimilar and otherwise highly persistent hydrocarbon compounds as well as the hyper-accumulation of heavy metal species. They show the development of techniques and protocols tailored to specific pollutants in both soils and waste waters with nuanced appreciation for the environmental conditions that may contribute or detract from their successful removal. Efforts are underway to examine and detail the mechanisms of removal at both the chemical and physiological levels. That the number of studies under review was nearly matched by the number of approaches and areas of interest suggests ample room for further research.

Bibliography

Abhilasha Shourie & U. Vijayalakshmi (2022) Fungal Diversity and Its Role in Mycoremediation, Geomicrobiology Journal, 39:3-5, 426-444, DOI: 10.1080/01490451.2022.2032883

Abiram Karanam Rathankumar, Kongkona Saikia, Krishnakumar Ramachandran, Ramon Alberto Batista, Hubert Cabana, Vinoth Kumar Vaidyanathan, Effect of soil organic matter (SOM) on the degradation of polycyclic aromatic hydrocarbons using Pleurotus dryinus IBB 903-A microcosm study, Journal of Environmental Management, Volume 260, 2020, 110153, ISSN 0301-4797, https://doi.org/10.1016/j.jenvman.2020.110153.

Ariste, Arielle Farida, Ramón Alberto Batista-García, Vinoth Kumar Vaidyanathan, Nikila Raman, Vasanth Kumar Vaithyanathan, Jorge Luis Folch-Mallol, Stephen A. Jackson, Alan D.W. Dobson, and Hubert Cabana. "Mycoremediation of Phenols and Polycyclic Aromatic Hydrocarbons from a Biorefinery Wastewater and Concomitant Production of Lignin Modifying Enzymes." *Journal of Cleaner Production* 253 (April 2020): 119810. https://doi.org/10.1016/j.jclepro.2019.119810.

Bartosz Kózka, Grzegorz Nałęcz-Jawecki, Jadwiga Turło, Joanna Giebułtowicz, Application of Pleurotus ostreatus to efficient removal of selected antidepressants and immunosuppressant, Journal of Environmental Management, Volume 273, 2020, 111131, ISSN 0301-4797, https://doi.org/10.1016/j.jenvman.2020.111131.

Benitez, S.F., M.A. Sadañoski, J.E. Velázquez, P.D. Zapata, and M.I. Fonseca. "Comparative Study of Single Cultures and a Consortium of White Rot Fungi for Polychlorinated Biphenyls Treatment." *Journal of Applied Microbiology* 131, no. 4 (October 2021): 1775–86. https://doi.org/10.1111/jam.15073.

Bhatt, M., Cajthaml, T. & Šašek, V. Mycoremediation of PAH-contaminated soil. Folia Microbiol 47, 255–258 (2002). https://doi.org/10.1007/BF02817647

Bhattacharya, S., Das, A., Prashanthi, K. et al. Mycoremediation of Benzo[a]pyrene by Pleurotus ostreatus in the presence of heavy metals and mediators. 3 Biotech 4, 205–211 (2014). https://doi.org/10.1007/s13205-013-0148-y

Bystrzejewska-Piotrowska, Grażyna, and Michał A. Bazała. "A Study of Mechanisms Responsible for Incorporation of Cesium and Radiocesium into Fruitbodies of King Oyster Mushroom (Pleurotus Eryngii)." Journal of Environmental Radioactivity 99, no. 7 (July 2008a): 1185–91. https://doi.org/10.1016/j.jenvrad.2008.01.016.

Bystrzejewska-Piotrowska, Grażyna, Dariusz Pianka, Michał A. Bazała, Romuald Stęborowski, José L. Manjón, and Pawel L. Urban. "Pilot Study of Bioaccumulation and Distribution of Cesium, Potassium, Sodium and Calcium in King Oyster Mushroom (*Pleurotus Eryngii*) Grown Under Controlled Conditions." *International Journal of Phytoremediation* 10, no. 6 (September 2, 2008b): 503–14. https://doi.org/10.1080/15226510802114987.

Chang, Bea-Ven, Shao-Ning Fan, Yao-Chou Tsai, Yi-Lin Chung, Ping-Xun Tu, and Chu-Wen Yang. "Removal of Emerging Contaminants Using Spent Mushroom Compost." *Science of The Total Environment* 634 (September 2018): 922–33. https://doi.org/10.1016/j.scitotenv.2018.03.366 .

Chang, Bea-Ven, Chiao-Po Yang, and Chu-Wen Yang. "Application of Fungus Enzymes in Spent Mushroom Composts from Edible Mushroom Cultivation for Phthalate Removal." *Microorganisms* 9, no. 9 (September 19, 2021): 1989. https://doi.org/10.3390/microorganisms9091989.

Chen, Miaomiao, Xin ZHENG, Liang CHEN, Xiaofang LI, Cadmium-Resistant Oyster Mushrooms from North China for Mycoremediation, Pedosphere, Volume 28, Issue 6, 2018, Pages 848-855, ISSN 1002-0160, https://doi.org/10.1016/S1002-0160(18)60047-5.

Chun, Se Chul, Manikandan Muthu, Nazim Hasan, Shadma Tasneem, and Judy Gopal. "Mycoremediation of PCBs by Pleurotus Ostreatus: Possibilities and Prospects." *Applied Sciences* 9, no. 19 (October 8, 2019): 4185. https://doi.org/10.3390/app9194185.

Dickson, Udeme & Coffey, Michael & Mortimer, Robert & Di Bonito, Marcello & Ray, Nicholas. (2019). Mycoremediation of Petroleum Contaminated Soils: Progress, Prospects and Perspectives. Environmental Science: Processes & Impacts. 21. 10.1039/C9EM00101H.

García-Delgado, Carlos, Irene Alfaro-Barta, and Enrique Eymar. "Combination of Biochar Amendment and Mycoremediation for Polycyclic Aromatic Hydrocarbons Immobilization and Biodegradation in Creosote-Contaminated Soil." *Journal of Hazardous Materials* 285 (March 2015): 259–66. https://doi.org/10.1016/j.jhazmat.2014.12.002.

Ibrahim, N. N., S. A. Talib, H. N. Ismail, and C. C. Tay. "Decolorization of Reactive Red-120 by Using Macrofungus and Microfungus." *Journal of Fundamental and Applied Sciences* 9, no. 6S (February 1, 2018): 954. https://doi.org/10.4314/jfas.v9i6s.11.

Kaewlaoyoong, Acharee, Jenq-Renn Chen, Chih-Yu Cheng, Chitsan Lin, Nicholas Kiprotich Cheruiyot, and Pongsert Sriprom. "Innovative Mycoremediation Technique for Treating Unsterilized PCDD/F-Contaminated Field Soil and the Exploration of Chlorinated Metabolites." *Environmental Pollution* 289 (November 2021): 117869. https://doi.org/10.1016/j.envpol.2021.117869.

Kapahi, Meena, and Sarita Sachdeva. "Mycoremediation Potential of Pleurotus Species for Heavy Metals: A Review." *Bioresources and Bioprocessing* 4, no. 1 (December 2017): 32. https://doi.org/10.1186/s40643-017-0162-8.

Kashangura, Chenjerayi, John E. Hallsworth, and Allen Y. Mswaka. "Phenotypic Diversity amongst Strains of Pleurotus Sajor-Caju: Implications for Cultivation in Arid Environments." *Mycological Research* 110, no. 3 (March 2006): 312–17. https://doi.org/10.1016/j.mycres.2005.10.006.

Leong, Yoong Kit, Te-Wei Ma, Jo-Shu Chang, and Fan-Chiang Yang. "Recent Advances and Future Directions on the Valorization of Spent Mushroom Substrate (SMS): A Review." *Bioresource Technology* 344 (January 2022): 126157. https://doi.org/10.1016/j.biortech.2021.126157.

Mayans, Begoña, Raquel Camacho-Arévalo, Carlos García-Delgado, Cynthia Alcántara, Norbert Nägele, Rafael Antón-Herrero, Consuelo Escolástico, and Enrique Eymar. "Mycoremediation of Soils Polluted with Trichloroethylene: First Evidence of Pleurotus Genus Effectiveness." *Applied Sciences* 11, no. 4 (February 3, 2021): 1354. https://doi.org/10.3390/app11041354.

Migliore, Luciana, Maurizio Fiori, Anna Spadoni, and Emanuela Galli. "Biodegradation of Oxytetracycline by Pleurotus Ostreatus Mycelium: A Mycoremediation Technique." *Journal of Hazardous Materials* 215–216 (May 2012): 227–32. https://doi.org/10.1016/j.jhazmat.2012.02.056.

Moeder, Monika, Tomáš Cajthaml, Gábor Koeller, Pavla Erbanová, and Václav Šašek. "Structure Selectivity in Degradation and Translocation of Polychlorinated Biphenyls (Delor 103) with a Pleurotus Ostreatus (Oyster Mushroom) Culture." *Chemosphere* 61, no. 9 (December 2005): 1370–78. https://doi.org/10.1016/j.chemosphere.2005.02.098.

Mohamadhasani, F., Rahimi, M. Growth response and mycoremediation of heavy metals by fungus *Pleurotus* sp.. *Sci Rep* **12**, 19947 (2022). https://doi.org/10.1038/s41598-022-24349-5

Mohammadi-Sichani, M., M. Mazaheri Assadi, A. Farazmand, M. Kianirad, A. M. Ahadi, and H. Hadian-Ghahderijani. "Ability of Agaricus Bisporus, Pleurotus Ostreatus and Ganoderma Lucidum Compost in Biodegradation of Petroleum Hydrocarbon-Contaminated Soil." International Journal of Environmental Science and Technology 16, no. 5 (May 2019): 2313–20. https://doi.org/10.1007/s13762-017-1636-0.

Njoku, K. L., Ulu, Z., Adesuyi, A. A., Jolaoso, A., & Akinola, M. (2018). Mycoremediation of Dichlorvos Pesticide Contaminated Soil by Pleurotus pulmonarius (Fries) Quelet. Pollution, 4(4), 605-615. doi: 10.22059/poll.2018.251177.385

Phan, Chia-Wei, and Vikineswary Sabaratnam. "Potential Uses of Spent Mushroom Substrate and Its Associated Lignocellulosic Enzymes." Applied Microbiology and Biotechnology 96, no. 4 (November 2012): 863–73. https://doi.org/10.1007/s00253-012-4446-9.

Pini, Andrea K., and Pamela Geddes. "Fungi Are Capable of Mycoremediation of River Water Contaminated by E. Coli." *Water, Air, & Soil Pollution* 231, no. 2 (February 2020): 83. https://doi.org/10.1007/s11270-020-4464-7.

Pozdnyakova, Natalia, Ekaterina Dubrovskaya, Dietmar Schlosser, Svetlana Kuznetsova, Elena Sigida, Vyacheslav Grinev, Sergei Golubev, Elena Kryuchkova, Giovanna Cristina Varese, and Olga Turkovskaya. "Widespread Ability of Ligninolytic Fungi to Degrade Hazardous Organic Pollutants as the Basis for the Self-Purification Ability of Natural Ecosystems and for Mycoremediation Technologies." *Applied Sciences* 12, no. 4 (February 18, 2022): 2164. https://doi.org/10.3390/app12042164.

Roshandel, Farzaneh, Sara Saadatmand, Alireza Iranbakhsh, and Zahra Oraghi Ardebili. "Mycoremediation of Oil Contaminant by Pleurotus Florida (P.Kumm) in Liquid Culture." *Fungal Biology* 125, no. 9 (September 2021): 667–78. https://doi.org/10.1016/j.funbio.2021.04.002.

Sadañoski, Marcela Alejandra, Silvana Florencia Benítez, María Isabel Fonseca, Juan Ernesto Velázquez, Pedro Darío Zapata, Laura Noemí Levin, and Laura Lidia Villalba. "Mycoremediation of High Concentrations of Polychlorinated Biphenyls with Pleurotus Sajor-Caju LBM 105 as an Effective and Cheap Treatment." *Journal of Environmental Chemical Engineering* 7, no. 6 (December 2019): 103453. https://doi.org/10.1016/j.jece.2019.103453.

Silva, André Felipe da, Ibrahim M. Banat, Diogo Robl, and Admir José Giachini. "Fungal Bioproducts for Petroleum Hydrocarbons and Toxic Metals Remediation: Recent Advances and Emerging Technologies." *Bioprocess and Biosystems Engineering*, August 9, 2022. https://doi.org/10.1007/s00449-022-02763-3.

Vaseem, Huma, V.K. Singh, and M.P. Singh. "Heavy Metal Pollution Due to Coal Washery Effluent and Its Decontamination Using a Macrofungus, Pleurotus Ostreatus." *Ecotoxicology and Environmental Safety* 145 (November 2017): 42–49. https://doi.org/10.1016/j.ecoenv.2017.07.001.

Wu, Minghui, Yongan Xu, Wenbo Ding, Yuanyuan Li, and Heng Xu. "Mycoremediation of Manganese and Phenanthrene by Pleurotus Eryngii Mycelium Enhanced by Tween 80 and Saponin." *Applied Microbiology and Biotechnology* 100, no. 16 (August 2016): 7249–61. https://doi.org/10.1007/s00253-016-7551-3.

Xu, Fei, Peng Chen, Hao Li, Suyu Qiao, Jiaxin Wang, Ying Wang, Xitong Wang, et al. "Comparative Transcriptome Analysis Reveals the Differential Response to Cadmium Stress of Two Pleurotus Fungi: Pleurotus Cornucopiae and Pleurotus Ostreatus." *Journal of Hazardous Materials* 416 (August 2021): 125814. https://doi.org/10.1016/j.jhazmat.2021.125814.

Yang, Chu-Wen, Ping-Hsun Tu, Wen-Yi Tso, and Bea-Ven Chang. “Removal of Organic UV Filters Using Enzymes in Spent Mushroom Composts from Fungicultures.” *Applied Sciences* 11, no. 9 (April 26, 2021): 3932. https://doi.org/10.3390/app11093932.

Zahmatkesh, Mostafa, Henri Spanjers, and Jules B. van Lier. “Fungal Treatment of Humic-Rich Industrial Wastewater: Application of White Rot Fungi in Remediation of Food-Processing Wastewater.” *Environmental Technology* 38, no. 21 (November 2, 2017): 2752–62. https://doi.org/10.1080/09593330.2016.1276969.