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# Mycoremediation: Expunging environmental pollutants

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ABSTRACT

The ever-increasing population, rapid rate of urbanization, and industrialization are exacerbating the pollution-related problems. Soil and water pollution affect human health and the ecosystem. Thus, it is crucial to develop strategies to combat this ever-growing problem. Mycoremediation, employing fungi or its derivatives for remediation of environmental pollutants, is a comparatively cost-effective, eco-friendly, and effective method. It has advantages over other conventional and bioremediation methods. In this review, we have elucidated the harmful effects of common pollutants on public health and the environment. The role of several fungi in degrading these pollutants such as heavy metals, agricultural, pharmaceutical wastes, including polycyclic aromatic hydrocarbons, is enumerated. Future strategies to improve the rate and efficiency of mycoremediation are suggested. The manuscript describes the strategies which can be used as a future framework to address the global problem of pollution.


## 1. Introduction

Pollution is a matter of grave concern. The poor management of the waste and effluents from households, industries, and agricultural fields is further deteriorating the already crippling ecosystem. According to the World Health Organization report, 2.2 billion people do not have access to safe water services, and 144 million people using contaminated water [1]. It is anticipated that by 2025, fifty percent of the world population will be living in the water-stressed regions [1]. In 1990, it was estimated that 22 million hectares of land were polluted, based on the current rate of urbanization and industrialization; this number is expected to increase further [2]. Pollution accounts for the loss of 5% gross domestic product (GDP) in developing countries [3]. It is accountable for 16 % of the global deaths, and 25 % of the most polluted regions [4]. Apart from its effect on public health and the economy, pollution can also threaten food security, drinking water availability, and biodiversity. So, it becomes imperative to develop strategies that can combat the soil and water pollution due to various chemicals and heavy metals. Currently, there are various physical and chemical methods available for the removal and degradation of various recalcitrant and harmful chemicals from soil and water. However, these methods are expensive, produce toxic byproducts, and ineffective for low concentrated but highly toxic chemicals [5]. A strategy has to be developed, which can overcome these limitations and provide *in situ* remediations of the pollutants.

Mycoremediation can be an economical, eco-friendly, and effective strategy to combat the ever-increasing problem of soil and water pollution. Robust growth of fungus, vast hyphal network, production of versatile extracellular ligninolytic enzymes, high surface area to volume ratio, resistance to heavy metals, adaptability to fluctuating pH and temperature and presence of metal-binding proteins; fungi are an ideal candidate for the remediation of various pollutants [5–8]. It can be used for the *in-situ* remediation of various pollutants such as dyes, herbicides and pharmaceutical drugs released by various industries. Alternatively, it can also be used in bioreactors. Bioreactors are systems with controlled physicochemical conditions aimed at promoting microbial growth [9]. Controlled fungal biomass and regulated use of metabolites in the bioreactor can be employed to accelerate the degradation of the pollutants [9–11]. So far, bioreactors are designed for the treatment of waste from sugar industries and pharmaceutical industries [9,12]. These bioreactors can be also be used *ex-situ* to remediate soil from polycyclic aromatic hydrocarbons, herbicides, pesticides, tars, chlorinated solvents and explosives [11].

In this review, the role of various fungi in degrading various recalcitrant, persistent and harmful pollutants like polycyclic

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aromatic hydrocarbon, pesticides, herbicides, insecticides, antifungal drugs, antibiotics, heavy metals, detergents, cyanotoxins, dyes, pharmaceuticals, and phthalates has been summarized. An attempt is made to understand the mechanism behind the mycoremediation of these pollutants. Future strategies to overcome the existing limitations and how the process of degradation can be accelerated is discussed.

## 2. Polycyclic aromatic hydrocarbons and their remediation

Polycyclic aromatic hydrocarbons (PAH) are one of the major pollutants that are released in the environment due to the incomplete combustion of organic materials like coal, wood, and petroleum. These toxic compounds can enter the air, water, and soil by various natural or human activities [13,14], (Fig. 1). PAH are organic compounds with fused benzene rings, namely pyrene, anthracene, chrysene, phenanthrene, 2-methyl naphthalene, acenaphthene. These ubiquitous pollutants can be classified into three categories: biological, petrogenic, and pyrogenic [15]. Exposure to these compounds can suppress immunity and cause cancers of skin, lungs, and stomach. They can also cause jaundice, inflammation, allergies, cataract, lysis of red blood cells, breathing problems, and can damage kidneys. Upon entering the environment, these chemicals will persist for prolonged duration and inflict severe damage to the ecosystem. Hence, the removal of these ubiquitous, persistent, toxic chemicals from contaminated soil and water seeks immediate attention [15]. For removing and degrading PAH from contaminated sites, various avenues like biodegradation, bioaccumulation, photodegradation, soil adsorption, leaching, and chemical oxidation have been extensively explored. Among these methods, biodegradation (use of microorganisms to degrade recalcitrant pollutants) is extensively researched [16]. However, the use of bacteria for degrading high molecular weight PAH has been observed to be a slow process due to the narrow degradation ability of these bacteria [17].

### *2.1. Role of the enzymes*

Ligninolytic enzymes released by several fungi are promising strategies in the removal of PAH. It is more economical and ecologically friendly than other conventional strategies to remove PAH from contaminated sites [12]. Some ligninolytic fungi have been shown to degrade both low and high molecular weight PAH that can consist of around six aromatic rings [16] (Table 1). These fungi secrete various extracellular ligninolytic enzymes, such as laccase, lignin peroxidase, and manganese peroxidase [13]. As these enzymes are promiscuous, they can also act on other substrates such as PAH, which are structurally similar to lignin [18]. Apart from the extracellular ligninolytic enzymes, cytochrome P-450 monooxygenase has also been shown to aid in PAH metabolism [19]. Recently, the role of non-ligninolytic enzymes has also been reported in degrading PAH. It has been shown that *Dentipellis* sp. *KUC8613* initially utilized cytochrome P450 followed by enzymes such as dioxygenase, dehydrogenases, FAD-dependent monooxygenases, glutathione transferase and epoxide hydrolases [20]. These extracellular ligninolytic enzymes catalyze the oxidation of PAH into quinones, which is then further degraded by ring fission [13,21]. Many of these fungi can completely breakdown PAH and produce $CO_2$, but certain ligninolytic fungi cannot degrade PAH completely [13]. In the latter case, the partially degraded PAH (into anthraquinone, diphenic acid, phthalic acid) is further decomposed by soil bacteria [13,22]. Though these fungi have shown the

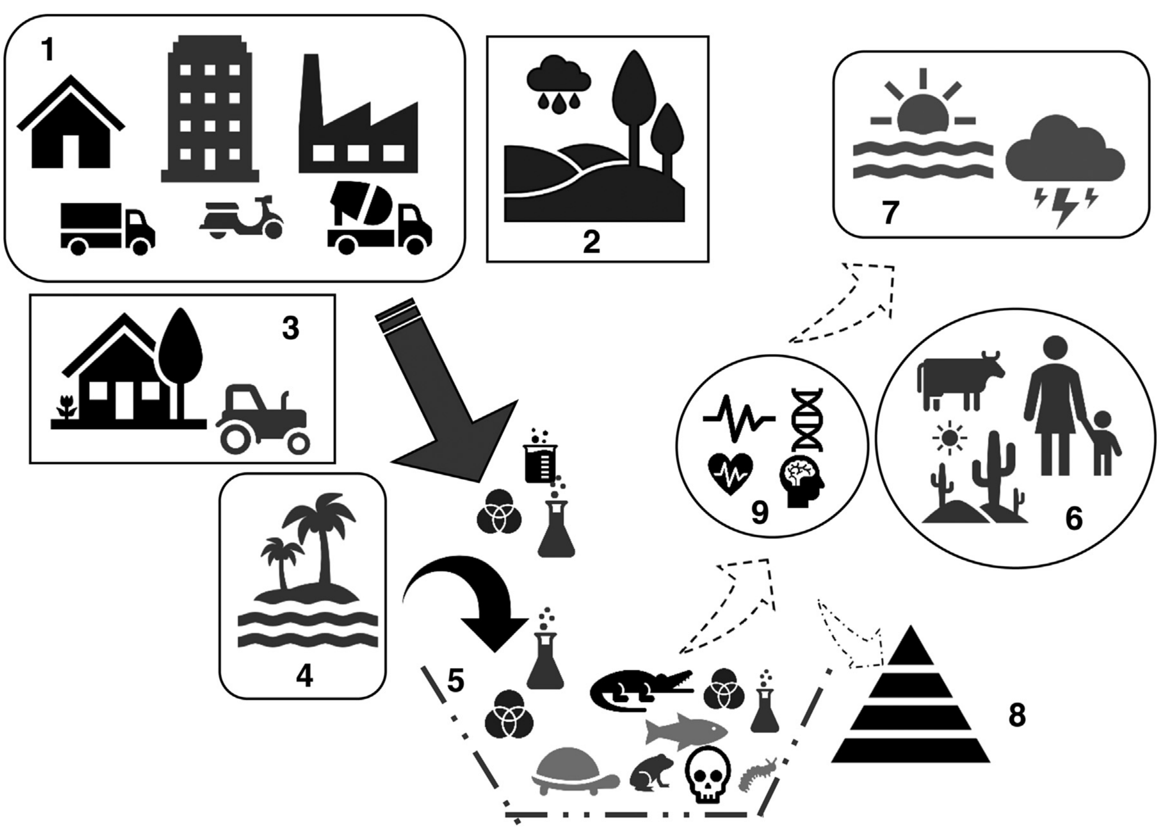


**Fig. 1.** Source of environment pollutants and its affect on health. Sources: (1) Rural and urban housing, industries, factories effluents, vehicular exhausts; (2) Soil pollution in dumpsites; (3) Agricultural wastes; (4) Beach pollution due to anthropogenic activities; Affect: (5) Water pollution, affecting aquatic life; (6) Humans, flora and fauna are affected (7) Biogeochemical cycles; (8) Accumulation of toxins in food chain; (9) Damage to overall health and well being.

**Table 1**
Source of common pollutants and fungi involved in its degradation.

| Pollutants | Fungi | Mechanism | References |
|---|---|---|---|
| Polycyclic aromatic hydrocarbon | *Dentipellis sp. (KUC8613), Phanerochaete chrysosporium, Trametes versicolor, Pleurotus ostreatus, Pleurotus eryngii, Cochliobolus lunatus* | Ligninolytic enzymes, cytochrome P-450 monooxygenase, dioxygenase, dehydrogenases, FAD dependent monooxygenases, glutathione transferase and epoxide hydrolases mediated degradation | [14,15,16,23,25] |
| Heavy metals | *Aspergillus species, Rhizomucor species, Fusarium* species, *Emericella species, Funneliformis geosporum, Pleurotus ostreatus, Trichoderma harzianum, Trichoderma ghanense, Penicillium rubens* | Ligninolytic enzymes in the degradation of heavy metals, and antioxidants enzymes in tolerating damage due to oxidative stress | [6,24,37,39,40] |
| Dyes | *Aspergillus flavus,* Marasmius cladophyllus, Phlebia acerina, Bjerkandera adusta | laccase, manganese peroxidase and lignin peroxidase in degradation of dyes | [8,89,90,91,92] |
| Pesticide and herbicide | *Botryosphaeria laricina*, *Aspergillus glaucus, Trametes pavonia, Penicillium spiculisporus, Penicillium verruculosum,* | Ligninolytic enzymes, esterification, dioxygenation, dehydrogenation, dechloriantion, demethylation mediated degradation | [42,43,45,46,47,48] |
| Antibiotics | *Pleurotus ostreatus, Leptospaherulina sp, Irpex lacteus, Lentinula edodes* | versatile peroxidase, laccase, manganese peroxidase, cytochrome 450 system | [71,72,73,74,76,77] |
| Pharmaceuticals | *Mucor hiemalis, Trametes versicolor, Phanerochaete chrysosporium, Lentinula edodes,* | ligninolytic enzymes and cytochrome 450 | [62,63,64,65,66,68] |
| Pthalates | *Fusarium oxysporum, Fusarium subglutinans,Penicllium brocae, Purpureocillium lilacinum* | Cutinase | [82,83,84,86,87,88] |
| Cyanotoxins and algal blooms | *Mucor hiemalis, Trametes versicolor, Trichoderma citrinoviride* | laccase, hydrolase, protease, cellulase and manganese peroxidase | [50,51,52,53,54,55] |
| Detergents | *Penicillium verrrucosum, Cladsporium cladosporioides* and *Geotrichum candidum* | NA[a] | NA |

[a] NA = Not applicable.

ability to degrade PAH, their efficiency is shown to be less due to inconsistent colonization and nutrient exhaustion. A study by Bhattacharya et al. showed that the biphasic treatment of PAH can overcome this limitation [19]. In the first phase *Phanerochaete chrysosporium*, a white rot fungus, were grown in under nutrient sufficient condition where the two genes (pc2 and pah4) coding for cytochrome P-450 monooxygenases were upregulated [19]. In the second phase the cells were exposed to nutrient deficient conditions which lead to the production of extracellular ligninolytic enzymes. This concerted effect of P-450 monooxygenases and ligninolytic enzymes was more efficient in degrading the PAH [19]. This also suggests that in earlier stages of PAH degradation there is a role of P-450 monooxygenases and in the later steps ligninolytic enzymes have an important role. This hypothesis needs to be studied further to formulate an efficient strategy to remove PAH from the environment.

### *2.2. Strategies to overcome the limitations of PAH degradation*

One of the limiting factors for the mycoremediation of PAH is the reduced bioavailability of PAH due to their hydrophobic nature and slow transport across the cell membrane [17]. Some fungi overcome this limitation by transporting the PAH to neighboring soil bacteria [17,23]. Other use hydrophobins to adsorbed these molecules [17]. Young et al. has shown more overexpression of a small hydrophobin in *Punctularia strigosozonata* during the degradation of hydrocarbons in fuel oil [24].

Similarly, the role hydrophobins have been reported in heavy metal biodegradation by *Trichoderma harzianum* [25]. However, during the degradation of PAH by *T. harzianum*, the hydrophobin gene *qid74* was not significantly upregulated [17]. Though studies suggest that the hydrophobins can help in degrading the PAH due to their ability to dissolve hydrophobic molecules [17], however, a conclusive study to corroborate this strategy has yet to be explored. The bioavailability of PAH has been observed to improve after the addition of surfactants like Tween-20, saponin, and Tween-80. These surfactants improve the solubility of the PAH [13]. The secretion of emulsifying agents by some fungi has also been reported. These naturally produced emulsifying agents in the presence of PAHs can further help to increase the bioavailability and degradation of various PAHs by elevating the solubility of PAH [26]. The degradation of PAH can also be increased by adding stimulants like cellulose and chitin. However, adding a higher concentration of these stimulants can decrease the degradation of PAH as these fungi use cellulose and chitin as a preferred carbon source [17]. Production of ligninolytic enzymes in these fungi occurs during secondary metabolite state. Hence, for the early synthesis of the ligninolytic enzymes, the cells should be deprived of carbon and nitrogen sources [27]. The addition of glycerol, veratrole alcohol, ferulic, humic acid, polyethylene glycol, coumaric acid, copper sulfate, vanillin, and citric acid has been shown to increase the expression of ligninolytic enzymes and degradation of PAH [13,16,27]. The addition of manganese and glutathione has also increased the activity and production of Manganese peroxidase [13].

The salinity of soil and the presence of heavy metals are other challenges for the degradation of PAH. In colder temperatures, the removal of the pollutant can be more challenging due to reduced moisture levels, slow metabolic process, and reduced available nutrients [28,29]. Moreover, cold temperatures can increase the viscosity of PAHs which will further decrease their bioavailability [28]. Cold environmental conditions can also thwart the activity of various enzymes imperative to degrade the toxic pollutants [28]. A study by Robichaud et al. showed that white-rot fungus *Trametes versicolor* which can degrade PAH was not able to grow at low temperatures at a contaminated site in Canada. However, fungi belonging to *Psathyrella* species were able to persist in such harsh arctic conditions in the presence of various pollutants [29]. The mycoremediation activity of *Psathyrella* species and other fungus is an exciting field for further research.

Heavy metals at the contaminated sites hamper the degradation owing to their toxicity towards ligninolytic fungi. They affect the structure and function of fungal cell membranes, energy transduction, metabolism, and growth of the fungi [30]. It also affects the activity of various ligninolytic enzymes [18]. However, some fungi like *Pleurotus ostreatus* and *Pleurotus eryngii* have been able to degrade PAH in the environment co-contaminated with heavy metals like cadmium, manganese, and mercury [18,30]. The activation of antioxidant enzymes helps these fungi to overcome free radicals stress caused by these heavy metals [31]. In another strategy, marine fungi can be used for the degradation. These fungi have an innate ability to tolerate high salt concentration. In this regard, a marine fungus, *Cochliobolus lunatus,* can be used to degrade PAH in soil and water with high salinity. *C. lunatus* has shown the ability to degrade chrysene [25]. The fungus was also able to tolerate low nutrient levels, varying temperatures, and pH.

Nevertheless, the study was not conducted in an area with high salinity. Future studies are required to evaluate the degradation potential of *C. lunatus* for PAHs in high salt concentration. A new strategy for the removal of PAH from soil can be its association with a plant. Mycoremediation of PAH by *Pleurotus ostreatus* in the presence of *Zea mays* helped in increasing manganese peroxidase activity, microbial, and fungal biomass [32]. The elevation in manganese peroxidase activity and fungal biomass can further increase the removal of these recalcitrant pollutants from the soil.

## 3. Heavy metal pollution and its mycoremediation

Heavy metal deposition in the environment poses a grave threat to biodiversity and human health. Arsenic, copper, lead, chromium, mercury, cadmium, silver, and nickel are some of the commonly found metals at the contaminated sites. These metals enter the soil and water by various human and natural activities (Fig. 1). Soil erosion, volcanic eruption, and weathering of earth's crust are some of the natural means of heavy metal pollution. The anthropogenic means include effluents from industries like paint, textile, metal parts, and fertilizer. Electronic wastes, leaded petrol, mining, preservatives, fungicides, insecticides, and combustion of fossil are other sources of heavy metals [33]. After leaching into the environment, these metals eventually enter the human body via the consumption of contaminated food and water [33]. At low concentration, some of these metals play an essential role in various metabolic and physiological activities, but above a threshold limit, they have deleterious effects [34]. Most of these metals are known as mutagens and carcinogens [35]. They also impair the proper functioning of the kidney, liver, spleen, heart and reproductive systems [36]. Apart from being a severe threat to public health, these metals also harm the ecosystem. Reactive oxygen species (ROS) generated due to these heavy metals oxidize various important biomolecules like nucleic acids and protein and lipids [37,38]. In microorganisms, these metals affect the activity of different enzymes like urease, catalase, glucosidase and alkaline phosphatase [39]. In plants, accumulation of these metals may hinder various physiological activities such as mineral and water transport and photosynthesis [40]. Other metals like mercury, lead, and arsenic can thwart the growth and metabolism, causes deformed fruits or flowers, hinder the growth of leaves, stem, and root; reduce crop yield, and $N_2$ fixing ability [40]. The oxidative stress due to heavy metals also causes lethal damage to aquatic life [41].

Various chemical and physical methods such as reverse osmosis, oxidation-reduction, filtration, ion exchange, chemical precipitation, and electrochemical treatment are available for the removal of heavy metals from the environment, but these methods have limitations. A few of the significant limitations of these strategies are high energy consumption, expensive, inefficient to remove metals at low concentration, and alteration of chemical properties of the soil [5]. Bioremediation can offer an efficient and inexpensive alternative to the conventional methods of substantial metal decontamination [42]. However, these metals have toxic effects on the plants beyond the threshold limit. Microorganism mediated bioremediation of heavy metals can be the answer to all the limitations discussed above and previous sections. Among all the microbes, fungi can be the most efficient removers of heavy metals due to the relatively higher tolerance to heavy metals, large surface to volume ratio, robust nature in comparison to bacteria and algae [5–7]. Biosorption, bioaccumulation, and biovolatilization are the principles often used by fungi to transform heavy metals and metalloids [43,44]. Biosorption is a passive (metabolically independent) process that can be performed by live cells or dead biomass, whereas bioaccumulation is active (metabolically dependent) process occurring in live cells [43,45]. Biovolatilization involves the conversion of inorganic and organic compounds into their volatile derivatives by intracellular enzymatic reactions [46]. Screening of sites contaminated with heavy metals has led to the identification of various fungi that can remove heavy metals. These indigenous fungi are not only resistant to heavy metal toxicity but are also adapted to the environmental condition of the contaminated site [5]. In one study, various fungi belonging to *Aspergillus, Rhizomucor, Fusarium* and *Emericella* species were isolated from agricultural land contaminated with arsenic (Table 1). On further analysis, it was found that these fungi were able to tolerate a high concentration of arsenic. Some of these fungi were also able to improve growth and yield in plants that were watered with sterile water containing arsenic. These fungi also improved soil enzyme activity and soil physio-chemical properties [6]. Another mycorrhizal fungus *Funneliformis geosporum* was able to improve the quality of soil and increase the growth and yield of the wheat crop in zinc contaminated soil by reducing the accumulation of zinc in the wheat plant [47]. This study suggests that these indigenous fungi not only remove heavy metals from the soil but also improve plant growth and crop yield in agricultural lands highly contaminated with heavy metals.

Similarly, *Pleurotus ostreatus* was also able to remove manganese from contaminated water in the presence of surfactants where these surfactants helped in increasing the surface area and metal-binding sites on fungal hypha eventually leading to bioaccumulation and efficient removal of manganese [30]. *Pleurotus ostreatus* was also able to remove heavy metals such as lead, zinc, chromium, cobalt, copper, and nickel present in effluents coming out of coal washery. There was an increased activity of antioxidant enzymes in the fungi due to the accumulation of heavy metals, which might have helped them to tolerate the toxic effects of heavy metals [31]. Further, different fungi such as *Fomitopsis meliae*, *Absidia cylindroslora Rhizopus microsporus,* and *Trichoderma ghanense* were also able to tolerate copper, cadmium, arsenic lead, and iron [48,49]. *Trichoderma harzianum,* isolated from an area surrounding an abandoned mine, was able to accumulate and tolerate silver [50].

Moreover, the native fungi growing in areas around gold, silver, and gemstones mine can accumulate gold and silver can offer an economic advantage [50]. Similarly, different *Aspergillus species* isolated from soil near an industrial area in Pakistan showed the ability to remove lead and mercury [35]. *Aspergillus species* and *Penicillium rubens* isolated from the same site were also able to remove Cadmium and Chromium [5]. Based on these studies, it can be concluded that the different indigenous fungi can be used for bioremediation of various toxic heavy metals and prevent the deleterious effect on the environment and human health. They can also improve the soil quality of agricultural land contaminated with heavy metals and enhance the growth and yield of different crops. However, for the actual removal of heavy metals, the remediating fungi should also be removed as the fungi tend to accumulate the metals within them. So, it is also imperative to develop strategies to overcome this limitation.

## 4. Agricultural effluents and their mycoremediation

Indiscriminate use of pesticides and herbicides is rampant in agrarian practices. These affect the soil groundwater or nearby water-bodies due to leaching of the minerals, like nitrogen, phosphorus, sulfur. It causes eutrophication by decreasing the dissolved oxygen and affect aquatic life. It also led to the accumulation of toxic chemicals in foods and vegetables, which enter the food chain and affect human health. The global market of the herbicides was USD 27.21 billion in 2016 and is projected to reach USD 39.15 billion by 2022. Herbicide and pesticide have helped the farmers across the globe in combatting various plant and insect diseases and in increasing the agricultural yield. On the contrary, these chemicals affect humans and cause severe damage to the ecosystem.

### 4.1. Mycoremediation of pesticides and herbicides

The chemicals present in the pesticides and herbicides like glyphosate, endosulfan, paraquat, fipronil, and aldrin are reported to be carcinogenic, endocrine disruptors, neurotoxic and cause lethal damages to the reproductive system and various vital organs like liver and kidney. Due to their deleterious side effects, most of these chemicals are either banned or heavily restricted [51,52]. However, despite such restrictions, the use of these harmful herbicides and pesticides is still prevalent. Moreover, chemicals like DDT, aldrin, and dieldrin can persist in the environment due to their chemical stability [53]. Most of these pesticides and herbicides runoff to nearby water sources or get accumulated in the soil and enter the food chain and affect microorganisms, plants, animals, and humans (Fig. 1) [51]. Different strategies involving chemical treatment, incineration and volatilization have been used for the degradation of these toxic compounds. However, these methods have certain limitations. Apart from cost and high energy consumption, these methods also release alkali, acids, and toxins into the environment. Further, for the treatment of the contaminated soil, the soil needs to be transported to a separate storage and treatment unit which further increases the cost and energy consumption [54]. So, mycoremediation seems like a plausible, eco-friendly, economical and in-situ strategy for the treatment of soil and water contaminated with herbicides and pesticides.

Various studies have shown the ability of different fungi to accumulate and remove herbicides and insecticides from the environment (Table 1). These studies were focused mostly on studying indigenous fungi growing in the contaminated sites. *Aspergillus tamarii* and *Botryosphaeria laricina* isolated from the agricultural field previously exposed to endosulfan were tolerant to endosulfan and were able to degrade the toxicant and its harmful metabolites like endosulfan sulfate, alpha endosulfan, and beta endosulfan by using them as a source of carbon and energy [55]. Similarly, in another study, it was found that a novel strain of *Aspergillus glaucus* was able to degrade fipronil and its metabolite fipronil sulfone [56] (Fig. 2). In this study, it was also reported that there was a role of different ligninolytic enzymes during the degradation of fipronil and its metabolite by *A. glaucus* [56]. Enzyme extracts from *Trametes maxima* and co-culture of *T. maxima* and *Paecilomyces carneus* were able to degrade 100 % of atrazine [57]. Camacho-Morales et al.; reported the possible involvement of different ligninolytic enzymes in the degradation of paraquat by *Trametes versicolor*, *Hypholoma dispersum* and *Trametes pavonia* [58]. Another xenobiotic compound glyphosate effectively degraded by fungus-like *Penicillium spiculisporus, Aspergillus flavus,* and *Penicillium verruculosum* which were isolated from soil samples taken from herbicide contaminated farms [54]. A white-rot fungus *Pleurotus ostreatus* was able to degrade aldrin and its major metabolite dieldrin. The biotransformation of aldrin and dieldrin by *P. ostreatus* was proposed to occur via epoxidation and hydroxylation reactions [53]. In some studies esterification, deoxygenation, dehydrogenation, dechlorination, demethylation reactions have been reported during the fungi mediated transformation of these pesticides [51]. Although some of the studies show the involvement of ligninolytic enzymes in the degradation of different herbicides and insecticides, a decisive role of these enzymes in degrading these xenobiotics is yet to be established. Furthermore, the mechanism used by these fungi in the biodegradation of xenobiotics is still not understood. Understanding the underlying mechanism can help in designing better strategies to combat the problem caused by these compounds.

### 4.2. Mycoremediation of cyanotoxins and algal blooms

Various anthropogenic activities are causing eutrophication that is responsible for the cyanobacteria bloom [59]. These cyanobacteria produce various secondary metabolites like cylindrospermopsin, Microcystin–LR, nodularians, and beta-*N*-methylamino-L-alanine [60]. These secondary metabolites are called cyanotoxins and can harm humans, animals, and aquatic life. In the aquatic system, they cause lethal poisoning in *Daphnia* species, affect photosynthesis of other cyanobacteria, reduce egg production in mollusks, suppress embryo development, and affect fishes (Fig. 1). These cyanotoxins are classified as neurotoxic, hepatotoxic, dermatologic to humans. They can also cause paralysis and allergic reactions in humans [59]. These cyanotoxins can also affect the farmers involved in pisciculture. To combat the problems caused by cyanotoxins mycoremediation can be useful. Aquatic fungi like *Mucor hiemalis* can accumulate and degrade cyanotoxins. It is

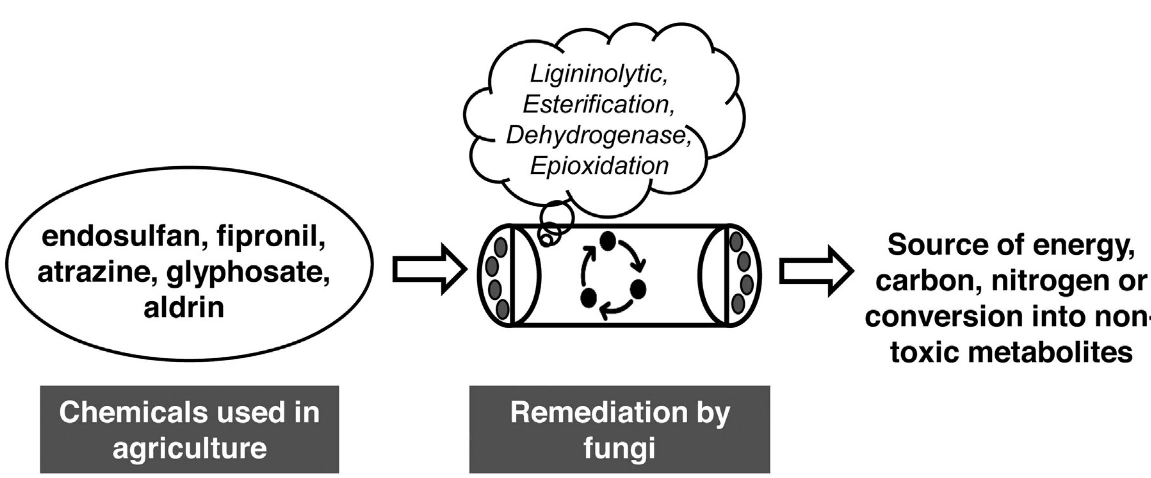


**Fig. 2.** Mycoremediation of herbicides and pesticides. The various chemicals used in agriculture are processed by fungi and are used as carbon or energy source or processed as non-toxic metabolites.

tolerant to oxidative stress due to cyanotoxins like Microcystin and beta-*N*-methylamino-L-alanine [60,61]. In the tolerance of oxidative stress, the role of various antioxidant enzymes was reported. However, the mechanism behind the degradation of these cyanotoxins is yet to be established. Different fungi like *Trametes versicolor* and *Trichoderma citrinoviride* has shown algicidal activity [62,63]. The transcriptomic and proteomic studies on these algicidal fungi have shown the role of various decomposition enzymes like laccase, hydrolase, protease, cellulase, and manganese peroxidase in killing and inhibiting the growth of various algae [62,64,65]. These studies suggest that mycoremediation can be a potential strategy to degrade cyanotoxins and inhibit the algal bloom formation.

## 5. Mycoremediation of pharmaceutical wastes

Pharmaceutical compounds released from the effluents of drug manufacturing units are exacerbating the already severed environmental conditions. The release of these compounds affects the public and animal health, disturbs the ecological balance, and further decreases the already dwindling available drinking water levels. It has been reported that in some of the cases, the environmental discharges consist of toxic threshold levels of drugs [66]. A study has shown that pharmaceutical effluents can have toxic effects on aquatic biota [67]. The commonly used water treatment systems fail to treat these xenobiotics, leading to the accumulation of the toxic pharmaceutical compounds in the environment [68].

Another widespread problem is antimicrobial resistance (AMR). Half a million people die globally due to the infections from drug-resistant microbes. The USA spent around 35 billion dollars in antimicrobial therapy [69]. These antibiotics can affect the ecologically important microorganisms that are imperative for the various processes like carbon cycling, soil respiration, iron reduction, denitrification, and nitrification [70]. Phytoremediation could be an alternative strategy. However, slow growth and longer remediation time limit the application of this strategy [42,71].

### 5.1. Mycoremediation of pharmaceuticals

Both aquatic and white-rot fungi can be promising candidates for the treatment of pharmaceutical wastes. Among these, the aquatic fungus *Mucor hiemalis* has been shown to remove the acetaminophen from contaminated water bodies of pharmaceutical effluents [68]. It was also able to uptake and remove another drug diclofenac [71]. The white-rot fungus *Trametes versicolor* was able to uptake and remove naproxen and its intermediates to undetectable levels within 6 h [72]. The *T. versicolor* treated media found to be nontoxic.

Similarly, *T. versicolor* was also able to degrade ketoprofen, an analgesic [73] (Table 1). In the degradation of naproxen and ketoprofen by *T. versicolor,* the role of ligninolytic enzymes and cytochrome 450 was reported [72,73]. *T versicolor* has also been able to degrade other pharmaceuticals like codeine, diazepam, carbamazepine, metoprolol, these studies were performed inside bioreactors [74]. *Phanerochaete chrysosporium* was able to perform oxidative degradation of different anti-inflammatory drugs inside the fed-batch stirred reactor [12]. The mycelia of edible fungus *Lentinula edodes* degraded anti-inflammatory drug piroxicam. *L. edodes* was also able to degrade endocrine disruptors like synthetic testosterone and 17α-ethyl-estradiol [75]. It secretes different enzymes with an oxidizing activity, which may be responsible for the oxidative degradation of piroxicam [76]. These studies show that the role of extracellular ligninolytic enzymes and intracellular cytochrome 450 systems are involved in the degradation of various pharmaceuticals. Studies have also shown the role of biosorption in the removal of pharmaceutical drugs [77]. In the future, bioreactors seem like a promising approach for the treatment of pharmaceutical waste [12,77]. However, the conditions for the better and efficient remediation of various pharmaceutical compounds from contaminated water and soil in fungal bioreactors need to be optimized.

### 5.2. Mycoremediation of antibiotics

Antibiotics are one of the major classes of drugs that are widely used across the globe, but unfortunately, many antibiotics are also being released into the environment through effluents from pharmaceutical industries, disposal of expired antibiotics, human feces and urine, hospital waste and careless use in animal husbandry and aquaculture [69,78]. The release of antibiotics in the environment can impact the economy, environment and public health [69,78]. The accumulation of antibiotics in water bodies can cause chronic toxicity in fishes, amphibians and other aquatic life forms [79]. In plants, the antibiotics affect electron transport rate, mitochondrial and chloroplast protein synthesis, inhibit plastid replication, decrease chlorophyll and carotenoid content. In plants, they also cause chromosomal aberrations and inhibit plant growth and photosynthesis [78]. Ozonation, oxidation using potassium permanganate, Fenton oxidation, sonolysis, photocatalysis using titanium dioxide, membrane bioreactors, and charcoal adsorption are some of the strategies used for the treatment of antibiotic contaminated water. But these processes are costly, energy-consuming and lead to the production of toxic byproducts [78]. Further, some antibiotics are resistant to the various physical and chemical methods due to their chemical structure [80]. Mycoremediation can be an economical, eco-friendly and safer alternative to various physical and chemical methods for the treatment of water contaminated with antibiotics.

Various fungi have been reported to eliminate different antibiotics like bifonazole, clotrimazole, sulfonamides, oxacillin, oxytetracycline, and fluoroquinolone [78,80–82], (Table 1). The ligninolytic fungi *Pleurotus ostreatus* absorbed oxytetracycline and eliminated the antibiotic from the liquid culture media within two weeks. There was no role of the ligninolytic enzyme laccase in the degradation of oxytetracycline [81]. However, in the degradation of different isoxazolyl-penicillins such as oxacillin and dicloxacillin by a Colombian strain of *Leptospaherulina sp*. the role of ligninolytic enzymes versatile peroxidase and laccase was reported [80]. The degradation of the antibiotics by *Leptospaherulina* sp. did not produce any toxic byproducts and there was also the elimination of the antibacterial of the isoxazolyl-penicillins. In the degradation of different fluoroquinolone antibiotics like ciprofloxacin and ofloxacin by *Trametes versicolor,* the role of ligninolytic enzymes was reported along with the cytochrome 450 system [83]. *T. versicolor* also eliminated sulfamethazine to undetectable levels [84]. The ligninolytic fungi *Irpex lacteus* degraded fluoroquinolone antibiotics like flumequine, ciprofloxacin, and ofloxacin effectively and quickly [85,86]. *Irpex lacteus* also removed the residual antibacterial activity of norfloxacin and ofloxacin, and in the degradation, there was the role of ligninolytic enzyme manganese peroxidase [85].

Similarly, the antifungal drugs bifonazole and clotrimazole were also absorbed and eliminated by the mycelia of the edible fungi *Lentinula edodes* [82]. Based on the studies reviewed here, it can be concluded that the mycoremediation can be a cheaper, effective, and safer strategy for the elimination of antibiotics from the environment. These studies also show that ligninolytic enzymes play a role in the degradation of antibiotics. However, the complete degradation pathway, biochemical and molecular mechanism needs to be studied. Optimum conditions, efficiency, and rate of antibiotic degradation should be explored for extensive scale remediation processes.

## 6. Mycoremediation of phthalates, dyes, and detergents

*Phthalates:* They are used in the production of vinyl tiles, capacitors, films, toys, and medical devices [87]. They provide flexibility to the plastics. However, their uncontrolled releases into the environment pave a path for these pollutants to enter the food chain, which eventually affects human health. In humans, they can reduce the level of testosterone, thyroid hormone, and pulmonary function. They are also a potent carcinogen and reduce male fertility [88–90]. Hydrolysis, UV irradiation, and titanium dioxide assisted photocatalytic degradation are some of the methods that can degrade the phthalates, but these processes are prolonged [87]. For the efficient and rapid degradation of phthalates, mycoremediation can be a good strategy.

Cutinase and esterase enzyme isolated from *Fusarium oxysporum* have been studied for their ability to degrade various phthalates like di-hexyl phthalate, dipropyl phthalate, di-2-ethylhexyl phthalate, butyl benzyl phthalate, and dipentyl phthalate. Cutinase was able to degrade these phthalates quickly and without the production of any toxic byproduct. However, the esterase mediated degradation is slow and produced byproducts that caused oxidative stress and affect enzyme activity [91–95]. Di-2-Ethylhexyl phthalate found in polyvinyl chloride blood storage bags was entirely degraded by *Purpureocillium lilacinum*, *Fusarium subglutinans, Aspergillus japonicas, Aspergillus parasiticus*, *Penicillium brocae* and *Penicillium funiculosum* isolated from soil samples obtained from sites heavily contaminated with plastics [96,97]. These studies show that mycoremediation can be an excellent strategy to combat phthalate pollution. The whole fungal cell biomass or isolated enzymes can be used for the large-scale purification of phthalate contaminated sites. However, the mechanism and genes involved in the degradation are yet to be established. The understanding of the degradation pathway at a biochemical and molecular level needs attention. Future studies should also focus on analyzing the potential of fungal biomass-based bioreactors for the degradation of phthalate. The optimization of conditions in bioreactors for rapid and efficient degradation of phthalates also needs to be studied.

*Dyes:* Millions of liters of wastewater with various dyes are released into the environment by many dyes producing industries, textile industries, and printing industries. These dyes are recalcitrant and persist in the environment for a long duration because due to inherent properties of these dyes to be chemically stable, resistant to fading, unaffected by microbial degradation and light. For the industries, it is cheaper and easier to release the effluents in the environment than to purify them [8]. Most of these effluents are toxic, mutagenic and carcinogenic [98]. It has been reported that fungi secreting lignin-degrading enzymes like laccase, manganese peroxidase, and lignin peroxidase can be able to degrade the dyes because of the non-specific activity of these enzymes [8,99].

*Aspergillus flavus* isolated from soil samples surrounding a paper processing industry was able to accumulate and eventually biodegrade Congo red dye [8]. In another study, Marasmius cladophyllus, an endophytic fungus isolated from Melastoma malabathricum, was also able to degrade different classes of synthetic dyes, but the mechanism and enzymes used by the fungi in degrading the dyes were not reported in the study [98]. Phlebia acerina was also able to clean up wastewater containing various dyes and further decreased the toxicity of the dye-containing wastewater [100]. Similarly, a strain of Tramtes versicolor was also able to degrade various azo anthraquinone type dyes [99]. Another

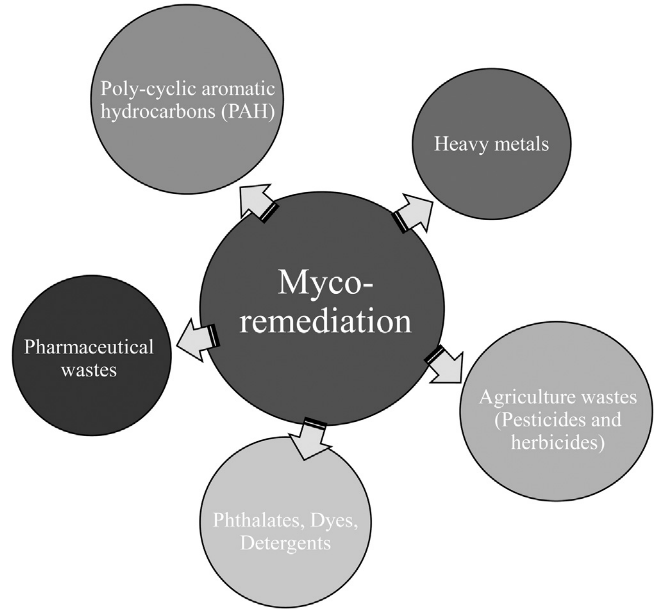


**Fig. 3.** Mycoremediation of environment pollutants.

fungus, Bjerkandera adusta, grown in an airlift bioreactor was able to decolorize different acid and reactive dyes at a quick rate [101]. In Tramtes versicolor, higher activity of ligninolytic enzymes like laccase and manganese peroxidase was reported and in Bjerkandera adusta higher activity of lignin peroxidase was reported [99,101]. The elevated level of the activity of the various lignin-degrading enzymes can help the fungi in degrading various dyes [99]. These studies show that various ligninolytic and endophytic fungi can be used as an economical, efficient and natural way of treating industrial effluents and soil containing a high amount of synthetic dyes. These fungi can tolerate heavy metals, act on diverse categories of dyes and do not produce any toxic secondary metabolites [8,99,101]. So, these studies corroborate that the mycoremediation can be a plausible solution for the treatment of synthetic dyes compared to other conventional physical and chemical methods.

*Detergents:* The release of the detergents in the environment is another serious matter of concern. Though these detergents are easily degradable and have no serious effect on public health, they can cause a great deal of harm to the aquatic flora and fauna. There are various methods to treat the wastewater contaminated with detergents, but compared to them, mycoremediation can be a cost-effective and efficient method. Different fungi like *Penicillium verrrucosum, Cladosporium cladosporioides,* and *Geotrichum candidum* have been able to degrade different commercially available detergents [102,103]. The mechanism of detergent degradation is yet to be explored. The molecular mechanisms including transcriptomic, proteomic and biochemical studies, should be done to elucidate the fungal detergent degradation pathway.

## 7. Conclusion

Based on the studies reviewed here, it can be concluded that mycoremediation can be an economical and effective strategy to degrade various recalcitrant, persistent and toxic pollutants like polyaromatic hydrocarbons, antibiotics, herbicides, insecticides, antifungal drugs, algal bloom, cyanotoxins, detergents, heavy metals, and plastic (Fig. 3). Most of the studies show the role of various extracellular ligninolytic enzymes and cytochrome 450 in the degradation of these pollutants. However, in most cases, the underlying mechanism of the mycoremediation of these harmful pollutants is elusive and needs further research. Functional or whole proteomic studies could be another area that should be explored to understand the mechanism of mycoremediation. Perhaps, these studies will reveal various genes and proteins involved in the mycoremediation process. The information will help to model genetically improved fungi for more efficient and rapid removal of the pollutants from the environment. Further, the fungi and their enzymes can be grown in bioreactors for the large-scale removal of the pollutants. There is a need for studies focusing on optimizing the conditions inside the bioreactors for the effective mycoremediation process. The indigenous fungi growing on polluted sites should be considered for further studies as they are adapted to the high concentration of various pollutants in harsh environmental conditions. Nevertheless, there is encouraging evidence to support that the mycoremediation can expunge the environmental pollutants and make this planet a safe habitat.

## Conflict of the interest

The authors have no conflict of interest to declare.

## CRediT authorship contribution statement

**Nahid Akhtar:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **M. Amin-ul Mannan:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.

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## References

[1] Drinking-water, (n.d.). https://www.who.int/news-room/fact-sheets/detail/drinking-water (Accessed 26 November 2019).

[2] N. Rodríguez Eugenio, F. Michael McLaughlin, Z. Bazza, F. Ronald Vargas, E. Kohlschmid, F. Oxana Perminova, F. Elisabetta Tagliati, A. Khan, L. Pennock, Hidden Soil Pollution a Reality Food and Agriculture Organization of the United Nations Edition, Design & Publication, 2018. (accessed November 26, 2019) www.fao.org/.

[3] V.R. Reddy, B. Behera, Impact of water pollution on rural communities: an economic analysis, Ecol. Econ. 58 (2006) 520–537, doi:http://dx.doi.org/10.1016/j.ecolecon.2005.07.025.

[4] P.J. Landrigan, R. Fuller, N.J.R. Acosta, O. Adeyi, R. Arnold, N.(Nil) Basu, A.B. Baldé, R. Bertollini, S. Bose-O'Reilly, J.I. Boufford, P.N. Breysse, T. Chiles, C. Mahidol, A.M. Coll-Seck, M.L. Cropper, J. Fobil, V. Fuster, M. Greenstone, A. Haines, D. Hanrahan, D. Hunter, M. Khare, A. Krupnick, B. Lanphear, B. Lohani, K. Martin, K.V. Mathiasen, M.A. McTeer, C.J.L. Murray, J.D. Ndahimananjara, F. Perera, J. Potočnik, A.S. Preker, J. Ramesh, J. Rockström, C. Salinas, L.D. Samson, K. Sandilya, P.D. Sly, K.R. Smith, A. Steiner, R.B. Stewart, W.A. Suk, O.C. P. van Schayck, G.N. Yadama, K. Yumkella, M. Zhong, The lancet commission on pollution and health, Lancet 391 (2018) 462–512, doi:http://dx.doi.org/10.1016/S0140-6736(17)32345-0.

[5] I. Khan, M. Aftab, S. Shakir, M. Ali, S. Qayyum, M.U. Rehman, K.S. Haleem, I. Touseef, Mycoremediation of heavy metal (Cd and Cr)–polluted soil through indigenous metallotolerant fungal isolates, Environ. Monit. Assess. 191 (2019), doi:http://dx.doi.org/10.1007/s10661-019-7769-5.

[6] M. Singh, P.K. Srivastava, P.C. Verma, R.N. Kharwar, N. Singh, R.D. Tripathi, Soil fungi for mycoremediation of arsenic pollution in agriculture soils, J. Appl. Microbiol. 119 (2015) 1278–1290, doi:http://dx.doi.org/10.1111/jam.12948.

[7] M. Kapahi, S. Sachdeva, Mycoremediation potential of *Pleurotus* species for heavy metals: a review, Bioresour. Bioprocess. 4 (2017), doi:http://dx.doi.org/10.1186/s40643-017-0162-8.

[8] S. Bhattacharya, A. Das, M. G, V. K, S. J, Mycoremediation of congo red dye by filamentous fungi, Braz. J. Microbiol. 42 (2011) 1526, doi:http://dx.doi.org/10.1590/S1517-838220110004000040.

[9] M.S. Aragão, D.B. Menezes, L.C. Ramos, H.S. Oliveira, R.N. Bharagava, L.F. Romanholo Ferreira, J.A. Teixeira, D.S. Ruzene, D.P. Silva, Mycoremediation of vinasse by surface response methodology and preliminary studies in air-lift bioreactors, Chemosphere 244 (2020)125432, doi:http://dx.doi.org/10.1016/j.chemosphere.2019.125432.

[10] S. Rodríguez Couto, A. Rodríguez, R.R.M. Paterson, N. Lima, J.A. Teixeira, Laccase activity from the fungus Trametes hirsuta using an air-lift bioreactor, Lett. Appl. Microbiol. 42 (2006) 612–616, doi:http://dx.doi.org/10.1111/j.1472-765X.2006.01879.x.

[11] M. Tekere, Microbial Bioremediation and Different Bioreactors Designs Applied, Biotechnol. Bioeng., IntechOpen, 2019, doi:http://dx.doi.org/10.5772/intechopen.83661.

[12] A.I. Rodarte-Morales, G. Feijoo, M.T. Moreira, J.M. Lema, Biotransformation of three pharmaceutical active compounds by the fungus Phanerochaete chrysosporium in a fed batch stirred reactor under air and oxygen supply, Biodegradation 23 (2012) 145–156, doi:http://dx.doi.org/10.1007/s10532-011-9494-9.

[13] N.N. Pozdnyakova, Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons, Biotechnol. Res. Int. 2012 (2012) 1–20, doi:http://dx.doi.org/10.1155/2012/243217.

[14] S.K. Samanta, O.V. Singh, R.K. Jain, Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, Trends Biotechnol. 20 (2002) 243–248, doi:http://dx.doi.org/10.1016/S0167-7799(02)01943-1.

[15] H.I. Abdel-Shafy, M.S.M. Mansour, A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation, Egypt. J. Pet. 25 (2016) 107–123, doi:http://dx.doi.org/10.1016/j.ejpe.2015.03.011.

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

[17] L.M. Czaplicki, M. Dharia, E.M. Cooper, P.L. Ferguson, C.K. Gunsch, Evaluating the mycostimulation potential of select carbon amendments for the degradation of a model PAH by an ascomycete strain enriched from a superfund site, Biodegradation 29 (2018) 463–471, doi:http://dx.doi.org/10.1007/s10532-018-9843-z.

[18] P. Baldrian, C. in Der Wiesche, J. Gabriel, F. Nerud, F. Zadrazil, Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil, Appl. Environ. Microbiol. 66 (2000) 2471–2478, doi:http://dx.doi.org/10.1128/aem.66.6.2471-2478.2000.

[19] S.S. Bhattacharya, K. Syed, J. Shann, J.S. Yadav, A novel P450-initiated biphasic process for sustainable biodegradation of benzo[a]pyrene in soil under nutrient-sufficient conditions by the white rot fungus *Phanerochaete chrysosporium*, J. Hazard. Mater. 261 (2013) 675–683, doi:http://dx.doi.org/10.1016/j.jhazmat.2013.07.055.

[20] H. Park, B. Min, Y. Jang, J. Kim, A. Lipzen, A. Sharma, B. Andreopoulos, J. Johnson, R. Riley, J.W. Spatafora, B. Henrissat, K.H. Kim, I.V. Grigoriev, J.-J. Kim, I.-G. Choi, Comprehensive genomic and transcriptomic analysis of polycyclic aromatic hydrocarbon degradation by a mycoremediation fungus, *Dentipellis* sp. KUC8613, Appl. Microbiol. Biotechnol. 103 (2019) 8145–8155, doi:http://dx.doi.org/10.1007/s00253-019-10089-6.

[21] K.E. Hammel, B. Green, Wen Zhi Gai, Ring fission of anthracene by a eukaryote, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10605–10608, doi:http://dx.doi.org/10.1073/pnas.88.23.10605.

[22] T.S. Brodkorb, R.L. Legge, Enhanced biodegradation of phenanthrene in oil tar-contaminated soils supplemented with *Phanerochaete chrysosporium*, Appl. Environ. Microbiol. 58 (1992) 3117–3121. (Sccessed 10 July 2019) http://www.ncbi.nlm.nih.gov/pubmed/1444426.

[23] S. Schamfuß, T.R. Neu, J.R. Van Der Meer, R. Tecon, H. Harms, L.Y. Wick, Impact of mycelia on the accessibility of fluorene to PAH-degrading bacteria, Environ. Sci. Technol. 47 (2013) 6908–6915, doi:http://dx.doi.org/10.1021/es304378d.

[24] D. Young, J. Rice, R. Martin, E. Lindquist, A. Lipzen, I. Grigoriev, D. Hibbett, Degradation of bunker C fuel oil by white-rot fungi in sawdust cultures suggests potential applications in bioremediation, PLoS One 10 (2015) e0130381, doi:http://dx.doi.org/10.1371/journal.pone.0130381.

[25] I. Puglisi, R. Faedda, V. Sanzaro, A.R. Lo Piero, G. Petrone, S.O. Cacciola, Identification of differentially expressed genes in response to mercury I and II stress in *Trichoderma harzianum*, Gene 506 (2012) 325–330, doi:http://dx.doi.org/10.1016/j.gene.2012.06.091.

[26] S.V. Nikiforova, N.N. Pozdnyakova, O.V. Turkovskaya, Emulsifying agent production during PAHs degradation by the white rot fungus *Pleurotus ostreatus* D1, Curr. Microbiol. 58 (2009) 554–558, doi:http://dx.doi.org/10.1007/s00284-009-9367-1.

[27] D. Singh, S. Chen, The white-rot fungus *Phanerochaete chrysosporium*: conditions for the production of lignin-degrading enzymes, Appl. Microbiol. Biotechnol. 81 (2008) 399–417, doi:http://dx.doi.org/10.1007/s00253-008-1706-9.

[28] K. Robichaud, C. Girard, D. Dagher, K. Stewart, M. Labrecque, M. Hijri, M. Amyot, Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North, Chemosphere 220 (2019) 47–55, doi:http://dx.doi.org/10.1016/j.chemosphere.2018.12.108.

[29] K. Robichaud, K. Stewart, M. Labrecque, M. Hijri, J. Cherewyk, M. Amyot, An ecological microsystem to treat waste oil contaminated soil: using phytoremediation assisted by fungi and local compost, on a mixed-contaminant site, in a cold climate, Sci. Total Environ. 672 (2019) 732–742, doi:http://dx.doi.org/10.1016/j.scitotenv.2019.03.447.

[30] M. Wu, Y. Xu, W. Ding, Y. Li, H. Xu, Mycoremediation of manganese and phenanthrene by *Pleurotus eryngii* mycelium enhanced by Tween 80 and saponin, Appl. Microbiol. Biotechnol. 100 (2016) 7249–7261, doi:http://dx.doi.org/10.1007/s00253-016-7551-3.

[31] H. Vaseem, V.K. Singh, M.P. Singh, Heavy metal pollution due to coal washery effluent and its decontamination using a macrofungus, *Pleurotus ostreatus*, Ecotoxicol. Environ. Saf. 145 (2017) 42–49, doi:http://dx.doi.org/10.1016/j.ecoenv.2017.07.001.

[32] Z. Košnář, T. Částková, L. Wiesnerová, L. Praus, I. Jablonský, M. Koudela, P. Tlustoš, Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relationto the extracellular enzyme activities, J. Environ. Sci. (China) 76 (2019) 249–258, doi:http://dx.doi.org/10.1016/j.jes.2018.05.007.

[33] S. Morais, F.G. e Costa, M. de Lourdes Pereir, Heavy metals and human health, Environ. Heal. - Emerg. Issues Pract., InTech, 2012, doi:http://dx.doi.org/10.5772/29869.

[34] M. Jaishankar, T. Tseten, N. Anbalagan, B.B. Mathew, K.N. Beeregowda, Toxicity, mechanism and health effects of some heavy metals, Interdiscip. Toxicol. 7 (2014) 60–72, doi:http://dx.doi.org/10.2478/intox-2014-0009.

[35] I. Khan, M. Ali, M. Aftab, S. Shakir, S. Qayyum, K.S. Haleem, I. Tauseef, Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metallotolerant fungi, Environ. Monit. Assess. 191 (2019), doi:http://dx.doi.org/10.1007/s10661-019-7781-9.

[36] V. Masindi, K.L. Muedi, Environmental contamination by heavy metals, Heavy Met., InTech, 2018, doi:http://dx.doi.org/10.5772/intechopen.76082.

[37] P.B. Tchounwou, C.G. Yedjou, A.K. Patlolla, D.J. Sutton, Heavy metal toxicity and the environment, EXS 101 (2012) 133–164, doi:http://dx.doi.org/10.1007/978-3-7643-8340-4_6.

[38] S.C. Gupta, D. Hevia, S. Patchva, B. Park, W. Koh, B.B. Aggarwal, Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy, Antioxid. Redox Signal. 16 (2012) 1295–1322, doi:http://dx.doi.org/10.1089/ars.2011.4414.

[39] M. Khan, Effect of metals contamination on soil microbial diversity, enzymatic activity, organic matter decomposition and nitrogen mineralisation (a review), Pak. J. Biol. Sci. 3 (2000) 1950–1956, doi:http://dx.doi.org/10.3923/pjbs.2000.1950.1956.

[40] G.U. Chibuike, S.C. Obiora, Heavy metal polluted soils: effect on plants and bioremediation methods, Appl. Environ. Soil Sci. 2014 (2014) 1–12, doi:http://dx.doi.org/10.1155/2014/752708.

[41] S. Gheorghe, C. Stoica, G.G. Vasile, M. Nita-Lazar, E. Stanescu, I.E. Lucaciu, Metals toxic effects in aquatic ecosystems: modulators of water quality, Water Qual., InTech, 2017, doi:http://dx.doi.org/10.5772/65744.

[42] C.C. Azubuike, C.B. Chikere, G.C. Okpokwasili, Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects, World J. Microbiol. Biotechnol. 32 (2016), doi:http://dx.doi.org/10.1007/s11274-016-2137-x.

[43] B.E. Igiri, S.I.R. Okoduwa, G.O. Idoko, E.P. Akabuogu, A.O. Adeyi, I.K. Ejiogu, Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review, J. Toxicol. 2018 (2018), doi:http://dx.doi.org/10.1155/2018/2568038.

[44] K. Boriová, S. Čerňanský, P. Matúš, M. Bujdoš, A. Šimonovičová, Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*, Lett. Appl. Microbiol. 59 (2014) 217–223, doi:http://dx.doi.org/10.1111/lam.12266.

[45] M. Fomina, G.M. Gadd, Biosorption: current perspectives on concept, definition and application, Bioresour. Technol. 160 (2014) 3–14, doi:http://dx.doi.org/10.1016/j.biortech.2013.12.102.

[46] M. Urík, S. Čerňanský, J. Ševc, A. Šimonovičová, P. Littera, Biovolatilization of arsenic by different fungal strains, Water Air Soil Pollut. 186 (2007) 337–342, doi:http://dx.doi.org/10.1007/s11270-007-9489-7.

[47] A.M. Abu-Elsaoud, N.A. Nafady, A.M. Abdel-Azeem, Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat, PLoS One 12 (2017)e0188220, doi:http://dx.doi.org/10.1371/journal.pone.0188220.

[48] O.G. Oladipo, O.O. Awotoye, A. Olayinka, C.C. Bezuidenhout, M.S. Maboeta, Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites, Braz. J. Microbiol. 49 (2018) 29–37, doi:http://dx.doi.org/10.1016/j.bjm.2017.06.003.

[49] Q. Albert, L. Leleyter, M. Lemoine, N. Heutte, J.P. Rioult, L. Sage, F. Baraud, D. Garon, Comparison of tolerance and biosorption of three trace metals (Cd, Cu, Pb) by the soil fungus *Absidia cylindrospora*, Chemosphere 196 (2018) 386–392, doi:http://dx.doi.org/10.1016/j.chemosphere.2017.12.156.

[50] G. Cecchi, P. Marescotti, S. Di Piazza, M. Zotti, Native fungi as metal remediators: silver myco-accumulation from metal contaminated waste-rock dumps (Libiola Mine, Italy), J. Environ. Sci. Health B 52 (2017) 191–195, doi:http://dx.doi.org/10.1080/03601234.2017.1261549.

[51] Z. Maqbool, S. Hussain, M. Imran, F. Mahmood, T. Shahzad, Z. Ahmed, F. Azeem, S. Muzammil, Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review, Environ. Sci. Pollut. Res. 23 (2016) 16904–16925, doi:http://dx.doi.org/10.1007/s11356-016-7003-8.

[52] A.S. Purnomo, T. Mori, K. Takagi, R. Kondo, Bioremediation of DDT contaminated soil using brown-rot fungi, Int. Biodeterior. Biodegrad. 65 (2011) 691–695, doi:http://dx.doi.org/10.1016/j.ibiod.2011.04.004.

[53] A.S. Purnomo, R. Nawfa, F. Martak, K. Shimizu, I. Kamei, Biodegradation of Aldrin and Dieldrin by the white-rot fungus *Pleurotus ostreatus*, Curr. Microbiol. 74 (2017) 320–324, doi:http://dx.doi.org/10.1007/s00284-016-1184-8.

[54] A. Eman, A. Abdel-Megeed, A.-M.A. Suliman, M.W. Sadik, E.N. Sholkamy, Biodegradation of Glyphosate by fungal strains isolated from herbicides polluted-soils in Riyadh area, (2013) . (Accessed 19 November 2019) http://www.ijcmas.com.

[55] S. Silambarasan, J. Abraham, Mycoremediation of endosulfan and its metabolites in aqueous medium and soil by botryosphaeria laricina JAS6 and *Aspergillus tamarii* JAS9, PLoS One 8 (2013), doi:http://dx.doi.org/10.1371/journal.pone.0077170.

[56] A. Gajendiran, J. Abraham, Biomineralisation of fipronil and its major metabolite, fipronil sulfone, by *Aspergillus glaucus* strain AJAG1 with enzymes studies and bioformulation, 3 Biotech 7 (2017), doi:http://dx.doi.org/10.1007/s13205-017-0820-8.

[57] W. Chan-Cupul, G. Heredia-Abarca, R. Rodríguez-Vázquez, Atrazine degradation by fungal co-culture enzyme extracts under different soil conditions, J. Environ. Sci. Health B 51 (2016) 298–308, doi:http://dx.doi.org/10.1080/03601234.2015.1128742.

[58] R.L. Camacho-Morales, K. Guillén-Navarro, J.E. Sánchez, Degradation of the herbicide paraquat by macromycetes isolated from southeastern Mexico, 3 Biotech 7 (2017), doi:http://dx.doi.org/10.1007/s13205-017-0967-3.

[59] G. Zanchett, E.C. Oliveira-Filho, Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects, Toxins (Basel) 5 (2013) 1896–1917, doi:http://dx.doi.org/10.3390/toxins5101896.

[60] E. Balsano, M. Esterhuizen-Londt, E. Hoque, S.P. Lima, Responses of the antioxidative and biotransformation enzymes in the aquatic fungus *Mucor hiemalis* exposed to cyanotoxins, Biotechnol. Lett. 39 (2017) 1201–1209, doi:http://dx.doi.org/10.1007/s10529-017-2348-8.

[61] Y. Jia, J. Du, F. Song, G. Zhao, X. Tian, A fungus capable of degrading microcystin-LR in the algal culture of *Microcystis aeruginosa* PCC7806, Appl. Biochem. Biotechnol. 166 (2012) 987–996, doi:http://dx.doi.org/10.1007/s12010-011-9486-6.

[62] W. Dai, X. Chen, X. Wang, Z. Xu, X. Gao, C. Jiang, R. Deng, G. Han, The algicidal fungus *Trametes versicolor* F21a eliminating blue algae via genes encoding degradation enzymes and metabolic pathways revealed by transcriptomic analysis, Front. Microbiol. 9 (2018), doi:http://dx.doi.org/10.3389/fmicb.2018.00826.

[63] Z.A. Mohamed, M. Hashem, S.A. Alamri, Growth inhibition of the cyanobacterium *Microcystis aeruginosa* and degradation of its microcystin toxins by the fungus *Trichoderma citrinoviride*, Toxicon 86 (2014) 51–58, doi:http://dx.doi.org/10.1016/j.toxicon.2014.05.008.

[64] X. Gao, C. Wang, W. Dai, S. Ren, F. Tao, X. He, G. Han, W. Wang, Proteomic analysis reveals large amounts of decomposition enzymes and major metabolic pathways involved in algicidal process of *Trametes versicolor* F21a, Sci. Rep. 7 (2017), doi:http://dx.doi.org/10.1038/s41598-017-04251-1.

[65] J. Du, G. Pu, C. Shao, S. Cheng, J. Cai, L. Zhou, Y. Jia, X. Tian, Potential of extracellular enzymes from *Trametes versicolor* F21a in *Microcystis* spp. degradation, Mater. Sci. Eng. C 48 (2015) 138–144, doi:http://dx.doi.org/10.1016/j.msec.2014.11.004.

[66] D.G.J. Larsson, Pollution from drug manufacturing: review and perspectives, Philos. Trans. R. Soc. B Biol. Sci. 369 (2014), doi:http://dx.doi.org/10.1098/rstb.2013.0571.

[67] C.G. Alimba, K.O. Adekoya, O.O. Soyinka, Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histopathological alterations in *Clarias gariepinus* (Burchell, 1822), EXCLI J. 18 (2019) 63–78. (Accessed 23 November 2019) http://www.ncbi.nlm.nih.gov/pubmed/30956640.

[68] M. Esterhuizen-Londt, K. Schwartz, S. Pflugmacher, Using aquatic fungi for pharmaceutical bioremediation: uptake of acetaminophen by *Mucor hiemalis* does not result in an enzymatic oxidative stress response, Fungal Biol. 120 (2016) 1249–1257, doi:http://dx.doi.org/10.1016/j.funbio.2016.07.009.

[69] S.A. Kraemer, A. Ramachandran, G.G. Perron, Antibiotic pollution in the environment: from microbial ecology to public policy, Microorganisms 7 (2019), doi:http://dx.doi.org/10.3390/microorganisms7060180.

[70] M. Cycoń, A. Mrozik, Z. Piotrowska-Seget, Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity, Front. Microbiol. 10 (2019), doi:http://dx.doi.org/10.3389/fmicb.2019.00338.

[71] M. Esterhuizen-Londt, A.L. Hendel, S. Pflugmacher, Mycoremediation of diclofenac using *Mucor hiemalis*, Toxicol. Environ. Chem. 99 (2017) 795–808, doi:http://dx.doi.org/10.1080/02772248.2017.1296444.

[72] E. Marco-Urrea, M. Pérez-Trujillo, P. Blánquez, T. Vicent, G. Caminal, Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR, Bioresour. Technol. 101 (2010) 2159–2166, doi:http://dx.doi.org/10.1016/j.biortech.2009.11.019.

[73] E. Marco-Urrea, M. Pérez-Trujillo, C. Cruz-Morató, G. Caminal, T. Vicent, White-rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR, Chemosphere 78 (2010) 474–481, doi:http://dx.doi.org/10.1016/j.chemosphere.2009.10.009.

[74] M.B. Asif, F.I. Hai, L. Singh, W.E. Price, L.D. Nghiem, Degradation of pharmaceuticals and personal care products by white-rot Fungi—a critical review, Curr. Pollut. Rep. 3 (2017) 88–103, doi:http://dx.doi.org/10.1007/s40726-017-0049-5.

[75] B. Muszyńska, P. Żmudzki, J. Lazur, K. Kała, K. Sułkowska-Ziaja, W. Opoka, Analysis of the biodegradation of synthetic testosterone and 17α-ethynylestradiol using the edible mushroom *Lentinula edodes*, 3 Biotech 8 (2018), doi:http://dx.doi.org/10.1007/s13205-018-1458-x.

[76] B. Muszyńska, M. Dąbrowska, M. Starek, P. Zmudzki, J. Lazur, J. Pytko-Polończyk, W. Opoka, Lentinula edodes mycelium as effective agent for piroxicam mycoremediation, Front. Microbiol. 10 (2019), doi:http://dx.doi.org/10.3389/fmicb.2019.00313.
[77] S. Yang, F.I. Hai, L.D. Nghiem, F. Roddick, W.E. Price, Removal of trace organic contaminants by nitrifying activated sludge and whole-cell and crude enzyme extract of Trametes versicolor, Water Sci. Technol. 67 (2013) 1216–1223, doi:http://dx.doi.org/10.2166/wst.2013.684.
[78] R. Gothwal, T. Shashidhar, Antibiotic pollution in the environment: a review, CLEAN Soil, Air, Water. 43 (2015) 479–489, doi:http://dx.doi.org/10.1002/clen.201300989.
[79] L. Liu, W. Wu, J. Zhang, P. Lv, L. Xu, Y. Yan, Progress of research on the toxicology of antibiotic pollution in aquatic organisms, Acta Ecol. Sin. 38 (2018) 36–41, doi:http://dx.doi.org/10.1016/j.chnaes.2018.01.006.
[80] L.S. Copete-Pertuz, J. Plácido, E.A. Serna-Galvis, R.A. Torres-Palma, A. Mora, Elimination of Isoxazolyl–Penicillins antibiotics in waters by the ligninolytic native Colombian strain *Leptosphaerulina* sp. considerations on biodegradation process and antimicrobial activity removal, Sci. Total Environ. 630 (2018) 1195–1204, doi:http://dx.doi.org/10.1016/j.scitotenv.2018.02.244.
[81] L. Migliore, M. Fiori, A. Spadoni, E. Galli, Biodegradation of oxytetracycline by *Pleurotus ostreatus* mycelium: a mycoremediation technique, J. Hazard. Mater. 215–216 (2012) 227–232, doi:http://dx.doi.org/10.1016/j.jhazmat.2012.02.056.
[82] A. Kryczyk-Poprawa, P. Żmudzki, A. Maślanka, J. Piotrowska, W. Opoka, B. Muszyńska, Mycoremediation of azole antifungal agents using in vitro cultures of Lentinula edodes, 3 Biotech 9 (2019), doi:http://dx.doi.org/10.1007/s13205-019-1733-5.
[83] A. Prieto, M. Möder, R. Rodil, L. Adrian, E. Marco-Urrea, Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products, Bioresour. Technol. 102 (2011) 10987–10995, doi:http://dx.doi.org/10.1016/j.biortech.2011.08.055.
[84] M.J. García-Galán, C.E. Rodríguez-Rodríguez, T. Vicent, G. Caminal, M.S. Díaz-Cruz, D. Barceló, Biodegradation of sulfamethazine by *Trametes versicolor*: removal from sewage sludge and identification of intermediate products by UPLC-QqTOF-MS, Sci. Total Environ. 409 (2011) 5505–5512, doi:http://dx.doi.org/10.1016/j.scitotenv.2011.08.022.
[85] M. Čvančarová, M. Moeder, A. Filipová, T. Cajthaml, Biotransformation of fluoroquinolone antibiotics by ligninolytic fungi—metabolites, enzymes and residual antibacterial activity, Chemosphere 136 (2014) 311–320, doi:http://dx.doi.org/10.1016/j.chemosphere.2014.12.012.
[86] M. Čvančarová, M. Moeder, A. Filipová, T. Reemtsma, T. Cajthaml, Biotransformation of the antibiotic agent flumequine by ligninolytic fungi and residual antibacterial activity of the transformation mixtures, Environ. Sci. Technol. 47 (2013) 14128–14136, doi:http://dx.doi.org/10.1021/es403470s.
[87] S. Benjamin, S. Pradeep, M. Sarath Josh, S. Kumar, E. Masai, A monograph on the remediation of hazardous phthalates, J. Hazard. Mater. 298 (2015) 58–72, doi:http://dx.doi.org/10.1016/j.jhazmat.2015.05.004.
[88] W.M. Kluwe, E.E. McConnell, J.E. Huff, J.K. Haseman, J.F. Douglas, W.V. Hartwell, Carcinogenicity testing of phthalate esters and related compounds by the National Toxicology Program and the National Cancer Institute, Environ. Health Perspect. 45 (1982) 129–133, doi:http://dx.doi.org/10.1289/ehp.8245129.
[89] T. Nagao, R. Ohta, H. Marumo, T. Shindo, S. Yoshimura, H. Ono, Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study, Reprod. Toxicol. 14 (2000) 513–532, doi:http://dx.doi.org/10.1016/S0890-6238(00)00105-2.
[90] R.W. Moore, T.A. Rudy, T.M. Lin, K. Ko, R.E. Peterson, Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer di(2-ethylhexyl) phthalate, Environ. Health Perspect. 109 (2001) 229–237, doi:http://dx.doi.org/10.1289/ehp.01109229.
[91] Y.H. Kim, H.S. Seo, J. Min, Y.C. Kim, Y.H. Ban, K.Y. Han, J.S. Park, K.D. Bae, M.B. Gu, J. Lee, Enhanced degradation and toxicity reduction of dihexyl phthalate by *Fusarium oxysporum* f. sp. pisi cutinase, J. Appl. Microbiol. 102 (2007) 221–228, doi:http://dx.doi.org/10.1111/j.1365-2672.2006.03095.x.
[92] Y.H. Kim, J. Min, K.D. Bae, M.B. Gu, J. Lee, Biodegradation of dipropyl phthalate and toxicity of its degradation products: a comparison of *Fusarium oxysporum* f. sp. pisi cutinase and *Candida cylindracea* esterase, Arch. Microbiol. 184 (2005) 25–31, doi:http://dx.doi.org/10.1007/s00203-005-0026-z.
[93] Y.H. Kim, J. Lee, S.H. Moon, Degradation of an endocrine disrupting chemical, DEHP [di-(2-ethylhexyl)- phthalate], by *Fusarium oxysporum* f. sp. pisi cutinase, Appl. Microbiol. Biotechnol. 63 (2003) 75–80, doi:http://dx.doi.org/10.1007/s00253-003-1332-5.
[94] Y.H. Kim, J. Lee, J.Y. Ahn, M.B. Gu, S.H. Moon, Enhanced degradation of an endocrine-disrupting chemical, butyl benzyl phthalate, by *Fusarium oxysporum* f. sp. pisi Cutinase, Appl. Environ. Microbiol. 68 (2002) 4684–4688, doi:http://dx.doi.org/10.1128/AEM.68.9.4684-4688.2002.
[95] J.Y. Ahn, Y.H. Kim, J. Min, J. Lee, Accelerated degradation of dipentyl phthalate by *Fusarium oxysporum* f. sp. pisi cutinase and toxicity evaluation of its degradation products using bioluminescent bacteria, Curr. Microbiol. 52 (2006) 340–344, doi:http://dx.doi.org/10.1007/s00284-005-0124-9.
[96] S. Pradeep, P. Faseela, M.K.S. Josh, S. Balachandran, R.S. Devi, S. Benjamin, Fungal biodegradation of phthalate plasticizer in situ, Biodegradation 24 (2013) 257–267, doi:http://dx.doi.org/10.1007/s10532-012-9584-3.
[97] S. Pradeep, S. Benjamin, Mycelial fungi completely remediate di(2-ethylhexyl)phthalate, the hazardous plasticizer in PVC blood storage bag, J. Hazard. Mater. 235–236 (2012) 69–77, doi:http://dx.doi.org/10.1016/j.jhazmat.2012.06.064.
[98] N.S. Ngieng, A. Zulkharnain, H.A. Roslan, A. Husaini, Decolourisation of synthetic dyes by endophytic fungal flora isolated from Senduduk Plant (*Melastoma malabathricum*), ISRN Biotechnol. 2013 (2013) 1–7, doi:http://dx.doi.org/10.5402/2013/260730.
[99] S.O. Yang, H. Sodaneath, J.I. Lee, H. Jung, J.H. Choi, H.W. Ryu, K.S. Cho, Decolorization of acid, disperse and reactive dyes by *Trametes versicolor* CBR43, J. Environ. Sci. Health A 52 (2017) 862–872, doi:http://dx.doi.org/10.1080/10934529.2017.1316164.
[100] R. Kumar, S. Negi, P. Sharma, I.B. Prasher, S. Chaudhary, J.S. Dhau, A. Umar, Wastewater cleanup using *Phlebia acerina* fungi: an insight into mycoremediation, J. Environ. Manage. 228 (2018) 130–139, doi:http://dx.doi.org/10.1016/j.jenvman.2018.07.091.
[101] H. Sodaneath, J.I. Lee, S.O. Yang, H. Jung, H.W. Ryu, K.S. Cho, Decolorization of textile dyes in an air-lift bioreactor inoculated with *Bjerkandera adusta* OBR105, J. Environ. Sci. Health A 52 (2017) 1099–1111, doi:http://dx.doi.org/10.1080/10934529.2017.1340753.
[102] V.D. Jakovljević, M.M. Vrvić, Potential of pure and mixed cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* for application in bioremediation and detergent industry, Saudi J. Biol. Sci. 25 (2018) 529–536, doi:http://dx.doi.org/10.1016/j.sjbs.2016.01.020.
[103] V.D. Jakovljević, M.M. Vrvić, *Penicillium verrucosum* as promising candidate for bioremediation of environment contaminated with synthetic detergent at high concentration, Appl. Biochem. Microbiol. 53 (2017) 368–373, doi:http://dx.doi.org/10.1134/S0003683817030164.