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Mycoremediation: a potential tool for sustainable management

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One of the major environmental problems faced today is the contamination of soil, water, and air by toxic chemicals. The distinct and unique role of microorganisms in the detoxification of polluted soil and environments is well recognized. Mycoremediation systems basically depend upon microorganisms (fungi) native to the contaminated sites. Fungi belonging to basidiomycetes are also known as mycoremediators because of their use in remediation of different types of pollutants. Mycoremediation relies on the efficient enzymes, produced by the fungus, for the degradation of various types of substrate and pollutants. However, sometimes they absorb the pollutant in their mycelium (biosorption) and cannot be consumed due to absorbed toxicants. Important fungi include *Pleurotus ostreatus, Rhizopus arrhizus, Phanerochaete chrysosporium, Tramates hirsute, T. versicolor, Lentinus edodes, Cladosporium resinae, Aspergillus niger, A. flavus, A. terreus and Trichoderma longibrachiatum. In-situ mycoremediation treats the contaminated soil before they can be treated. However, despite being the living dominating biomass in the soil, fungi have not yet been significantly exploited for mycoremediation of such polluted environments. More extensive research needs to be carried out on the use of fungi in mycoremediation.*

Key words: detoxification, mycoremediation, biosorption, excavation

INTRODUCTION

Soil pollution has significant deleterious consequences for the ecosystem. In the past few years, the soil is getting more and more polluted. Remediation of these polluted soils is a challenging job. Bioremediation is a treatment process that uses naturally occurring microorganisms to break down, or degrade, hazardous substances into less toxic or nontoxic substances. The microbes used to perform the function of bioremediation are known as bioremediators. To "bioremediate", means to use living things to solve an environmental problem such as contaminated soil or groundwater. The introduction of exogenous microorganisms into environments bioaugmentation, has been used as an attempt to accelerate bioremediation Some microorganisms that live in soil and groundwater naturally degrade certain chemicals that are harmful to people and the environment. These microorganisms are also able to change these chemicals into water and harmless gases, such as carbon dioxide etc. Plants can also be used to clean up soil, water or air; this is called *phytoremediation*. It is also reported that naturally occurring microbial consortia (bacteria/ fungi) have been utilized in a variety of bioremediation processes.

Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to the less hazardous or non-hazardous forms with less input of chemicals, energy and time. During the last two decades, many mycologists have tried the use of various fungal species in the degradation of organic compounds. The discovery of the white rot fungi (*Phanerochaete chrysosporium*) in bioremediation has brought greater success and thus initiated the research throughout the world on mycoremediation, establishing the fact that fungi can be successfully used in bioremediation.

The term mycoremediation can be broken down as myco (fungus) and remediation (to clean, resolve, or correct), and indeed, mycoremediation is the use of fungi, specifically mushrooms, for

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creating simple yet effective biomass capable of breaking down environmental and industrial pollutants. The mycelium is a sort of self-healing filter that targets specific organic compounds and pollutants. Research has proven the efficacy of using fungi to degrade contamination such as PCBs, aromatic hydrocarbons, and oil spills. Biological pollutants, especially *E. coli*, have been of special interest in recent years, and a wealth of data now supports the benefits of mycoremediation in reducing or eliminating such pathogenic organisms. So, mycoremediation is described as a form of bioremediation in which more contaminated sites are converted into less contaminated sites by the use of fungi. Mycelium stimulates microbial and enzyme activity and thus reduces in-situ production of toxins (Adenipekun and Lawal, 2012). The potential applications for mycoremediation technologies have been reported from time to time. Fungi have been shown to accumulate toxic metals and even rare earth elements. Fungi are great biodegraded and the resultant compost has been used to enhance the growth of plants as well as bioremediation activity in the environment. Mycelia of fungi are unique among microorganism having the ability to enhance plant growth. They secrete a variety of extracellular enzymes involved in pollutants degradation. Some fungi are hyper-accumulators and are capable of absorbing and concentrating heavy metals in the fruiting bodies of mushrooms (Adenipekun and Lawal, 2012).

Mycoremediation practices involve mixing of mycelium (the vegetative part of a fungus) into contaminated soil; placing mycelial mats over toxic sites; or and even the combination of these two techniques. Mycoremediation has been applied to oil spills, contaminated and polluted soil, industrial chemicals, contaminated water and even farm waste . They reported that bioremediation technology leads to the degradation of pollutants and maybe a lucrative and environmentally beneficial alternative. Many reports have published to emphasize the role of mushroom in bioremediation of wastes by the process of biodegradation, biosorption, and bioconversion. The mushroom can produce extracellular peroxidases, ligninase (lignin peroxidase, manganese-dependent peroxidase, and laccase), cellulases, pectinases, xylanases and oxidases (Kulshrestha et al. 2014; Majeau et al. 2010; Ruiz-Duenas et al. 2008 ; Hofrichter et al. 2010).

These are able to oxidize recalcitrant pollutants in vitro. These enzymes are typically induced by their substrates. These enzymes have also been found to degrade non-polymeric, recalcitrant pollutants such as nitrotoluenes PAHs organic and synthetic dyes, and pentachlorophenol under in vitro conditions. Recently, it is reported that mushroom species are able to degrade polymers such as plastics. The biodegradation mechanism is very complex. The reason is the influence of other biochemical systems and interactions of lignolytic enzymes with cytochrome P450 monooxygenase system(Cerniglia and Sutherland, 2010; Kasai, N. et al. 2010; Subramanian and Yadav 2009), hydroxyl radicals and the level of H?O? which are produced by the mushroom.

ROLE OF FUNGI IN MYCOREMEDIATION

Fungi have been harnessed in many diverse applications for thousands of years ago. In an ecosystem, they are among the major decomposers of various complex polymers as cellulose, hemicelluloses, and lignin etc. Fungi have the ability to store, release various elements and ions and they can even accumulate toxic elements An edible and medicinal fungus also plays an important role as natural environment remediator. The goal of mycoremediation is to stimulate microorganisms with nutrients and other chemicals that will enable them to destroy the contaminants. Mycoremediation is an innovative biotechnological application that uses living fungus for in situ and ex-situ cleanup and management of contaminated sites (Thomas et al. 2009).

Mycoremediation is not widely used at present, but the above applications suggest its broader potential. Fungi perform a wide variety of functions in the ecosystem and maybe a clean, simple and relatively inexpensive method of environmental remediation, especially if species native to each site is used. Mycoremediation is a form of bioremediation that uses native fungi and fungal mycelium applied to surface soils to remove and degrade contaminants (Thomas *et al.* 2009).

MYCOREMEDIATION AND XENOBIOTICS

Contamination of soil and water by toxic pollutants is a worldwide problem. These contaminants include Petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), halogenated organic compounds, dyes, nitrogen-containing xenobiotics, pesticides and inorganic pollutants (heavy metals). These chemicals are called xenobiotics [G. xenos = foreigner, stranger; bios = life] since these compounds differ substantially in chemical structure from natural organic compounds and these are relatively recalcitrant to biodegradation. Certain substituents such as halogen, sulfo-, azo- or nitro-groups, particularly the accumulation of such groups and specific substitution patterns, confer xenobiotic character to a synthetic compound. Moreover, the electronwithdrawing character of these substituents generates an electron deficiency and thus makes the compounds less susceptible to oxidative catabolism. The contamination of soil due to toxic pollutants is classified as following

Petroleum hydrocarbons (PHs):

Petroleum hydrocarbons contain a complex mixture of compounds that can be categorized into four fractions: saturates, aromatics, asphaltenes, and resins(Das and Chandan, 2011).The saturated fraction consists of straight-chain alkanes (normal alkanes), branched alkanes (isoalkanes), and cycloalkanes (naphthenes). The aromatic fraction includes volatile monoaromatic hydrocarbons such as benzene, toluene, and xylenes; polyaromatic hydrocarbons (PAH) such as naphthene aromatics; and aromatic sulfur compounds, such as thiophenes and dibenzothiophenes. The asphaltene (phenols, fatty acids, ketones, esters, and porphyrins) and resin (pyridines, quinolines, carbazoles, sulfoxides, and amides) fractions consist of polar molecules containing N, S, and O?. Asphaltenes are large molecules dispersed in oil in a colloidal manner, whereas resins are amorphous solids truly dissolved in oil (Table 1).

Polycyclic aromatic hydrocarbons (PAHs)

PAHs are released into the environment as a result of a variety of activities, such as incomplete combustion of fossil fuels, shale oil, and cigarette smoke; accidental discharge of petroleum or during the use and disposal of petroleum products; and coal gasification and liquefaction (Fig 1; Table 1).

Halogenated organic compounds (HOCs):

The relatively great electronegativity of halogens confers chemical stability of the compound, making

these recalcitrant to biodegrading. Halogen substituents can increase the hydrophobicity of the compounds, increasing their tendency to bioaccumulate in food chains as well as to sorb to the soil. Finally, the halogen substituents can contribute to harmful biological effects of the compounds, increasing their toxicity, mutagenicity and other detrimental capacities. Some xenobiotic halogenated organic compounds are pentachlorophenol (PCP), trichloroethene (TCE), 2, 4-dichlorophenoxyacetic acid (2, 4-D), polychlorinated biphenyl (PCB), dioxins (Table 1).

Dyes

Synthetic dyes are employed increasingly in the textile, paper, cosmetic, pharmaceutical, and food industries, due to high stability, a wide variety of color, and good cost-effectiveness in synthesis compared to natural dyes. These are also used in printing industries, color photography, and as additives in petroleum products. Environmental control of dyes is important due to their possible toxicity and carcinogenicity (Table 1).

Pesticides

Synthetic pesticides have been known since 1939 when the insecticidal properties of DDT were discovered. Pesticides are used extensively related to agriculture, animals, and humans to protect public health. The extensive use of pesticides has contributed to the contamination of many terrestrial and aquatic global ecosystems due to their extreme toxicity and persistence in the environment. Based on their applications in agriculture, animals, and humans, pesticides are divided into three categories: insecticides, herbicides, and fungicides are further classified into different categories based on chemical composition and organic grouping (Table 1).

Heavy metal toxicity and its sources

The term heavy metals strictly refers to metallic elements which have a specific mass > 5 gcm-³ and able to form sulfides. Zn, Cu, Mn, Ni, and Co are essential nutrients and are toxic at high concentration; Cd, Pb, As and Hg are nonessential with no known biological function and are toxic at low concentration. After entering within the cell through specific uptake system, heavy-metal

Tremates versicolor, Rhodotorula rubra Phanerochaete chrysosporium Trametes versicolor, Pleurotus ostreatus Bjerkandera sp. Phanerochaete chrysosporium <u>miniodsos/</u> Phanerochaete chrysosporium White Rot Fungi Pleurotus pulmonarius Polyporus ostreiformis Pleurotus ostreatus Phanerochaete chr Crinipellis stipitaria Cariolus versicolor Fungi involved in mycoremediation Aspergillus terreus Curningharnella elegans Aspergillus niger Penicillium sp. Filamentous Fungi Cladosporium resinae Cunninghamella elegans Fusarium solani Cunninghamella elegans Penicilliumm egasporum Trichoderma harzianum Aspergillus terreus Aspergillus flavus Pyriculari aoryzae H Candida lipolytica C. tropicalis Graphium putredinis Candida lipolytica Yeast Azure B, Orange II, Tropacolin O, Congo Red, Rosc Bengal Edifenphos GROUP 2,4-D DDT Remazol Brilliant Blue R Malachite Green **Crystal violet** Congo red Name of xenobiotic compounds Insecticides Herbicides Fungicide Halogenated organic compounds (HOCs) Polycyclic aromatic hydrocarbons (PAHs) NAME Petroleum hydrocarbons (PHs) Pesticides Dyes

Table 1: List of some fungi (both filamentous and white-rot fungi) involved in mycoremediation

On mycoremediation

[J. Mycopathol. Res. :



Fig. 2 : List of some commonly occurring white-rot fungi involved in mycoremediation

Name	Role in mycoremediation	
Genus- <i>Agaricus</i> A. <i>augustus</i> (the prince) A. <i>bisporus</i> (button) A. <i>campestris</i> (forest mushroom) A. <i>blazei</i> (almond scented agarid)	Mushrooms in the genus <i>Agaricus</i> have been shown to hyper-accumulate cadmium, copper, and zinc. The almond-flavored agarics in particular also contain high levels of antibiotic properties.	
Genus- <i>Agrocybe</i> A. <i>aegerit</i> a (black poplar) A. <i>cylindrace</i> a (pioppino) A. <i>praecox</i> (swordbelt)	Mushrooms in this genus are known for their wide spectrum of activity based on laccase, an oxide reductase enzyme. They are known for their ability to break down endocrine disruptors, like BPA, and could be useful for mycofiltration projects.	
Genus-A <i>uricularia</i> A. <i>auricula</i> , A. <i>fuscosuccinea</i> , A. <i>polytrich</i> a (wood ear, tree ear)	Auricularia mushrooms may be best used for habitat renewal, particularly for devastated ecosystems that are rich in lignicolous debris, such as logged forests. Given their ability to thrive with more sun exposure than other wood -decomposing mushrooms, these fungi can be useful to help build soil in arid, sunny environments.	
Genus- <i>Clitocybe</i> <i>C. nud</i> a (blewit, wood blewit) <i>C. saeva</i> (blue legs)	Some studies suggest that extracts from the fruitbody of blewits can be used as a foliar bactericide and insecticide. Blewits are also well known for their ability to hyperaccumulate metals in their mycelium, making them well suited for mycofiltration of contaminated soil and water.	
Genus- <i>Coprinus</i> C. <i>comatus</i> (shaggy mane, inky cap)	<i>Coprinus</i> species are efficient hyperaccumulators of mercury, cadmium, and arsenic. Enzymes from this mushroom, curiously, have been used as a dye remediation additive, and some studies have used them in detergents for washing clothes. Living mycelium from this fungus also attacks nematodes.	
Genus- <i>Fistulina</i> <i>F. hepatica</i> (beefsteak)	This mushroom is commonly found in logged sites, even in full-sun environments, making it a good candidate for landscape remediation in logged areas where most other fungi will not thrive.	
Genus- <i>Flammulin</i> a <i>F. populicola,</i> <i>F. velutipes</i> (enoki, velvet foot)	Enoki is a well -known stump recycler, giving it a possible advantage if used to remediate a logging site, perhaps along with compatible species such as beefsteak polypore and wood ear mushrooms, which also can grow in these otherwise fungus-inhospitable locations.	
Genus- <i>Ganoderma</i> G. applanatum (artist conk) G. curtisii, G. Iucidum,	Reishi mycelium has an extremely high tensile strength and an affinity for inhibiting and lysing bacterial cells, making it a good candidate for mycofiltration of water.	
<i>G. oregononso,</i> <i>G. resinaceum</i> (reishi, lirg chi) <i>G. tsugae</i> (hemlock reishi)		

cations, such as Hg²⁺ and Cd²⁺ tend to bind to SH groups and inhibit the activity of sensitive enzymes. Other heavy-metal cations may interact with physiological ions, thereby inhibiting the function of the respective physiological cation. The sources of the metals in the soil are diverse, including the burning of fossil fuels, mining, and smelting of metalliferous ores, municipal wastes, fertilizers. pesticides. sewage sludge amendments, effluents from industries like electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc. To survive under metal-stressed environment, microorganisms have evolved several mechanisms (Fig 3). They can change or reduce the toxicity of metallic contaminants through pH change, biosorption and bioaccumulation. Biosorption consists of a metabolism-independent



Fig.1: Three major pathways of degradation of polycyclic aromatic hydrocarbons (PAHs) by fungi and bacteria

binding of metal ions to negatively charged free groups in several biopolymers that form the microbial cell wall whereas bioaccumulation employs an energy-dependent metal influx. They produce intracellular/extracellular enzymes to resist the metal concentration or they possess the processes of active transport of metal ions outside the cell, masking metals by chelating, enzymatic transformation of metal ions, creating vacuoles in which metal ions are gathered and immobilization in the form of polyphosphates, increased production of melanin and other pigments, and production of specific metal-binding compounds (e.g. metallothioneins) inside the cell. Biovolatilization is an enzymatic conversion of organic and inorganic compounds of metal (loid) s into their volatile derivatives by an intracellular biochemical reaction (biomethylation) (Table 1).



HOW DOES MYCOREMEDIATION WORK?

In order for the fungal cultures to do their work, the extrinsic and intrinsic growth factors viz., right temperature, nutrients and amount of oxygen must be present in the soil and groundwater. The right combinations of these cultures can eat the pollutants until they disappear. After the process of remediation is over, the fungal mycelia themselves disappear because there's no more pollution for them to eat. Fungi are proficient bioremediators by breaking down long chained toxins into simpler less toxic chemicals. They remove metals from land by channeling them to mushroom fruiting bodies for removal. They essentially use and digest these toxins as nutrients. Even the enzymes secreted from mycelium can decompose some of the most resistant hazardous toxin materials made by humans or nature. These toxins are vulnerable to enzymes secreted by the mycelia. Fungi possess the biochemical and ecological capacity to degrade

environmental organic chemicals and to decrease the risk associated with metals, metalloids, and radionuclides, either by chemical modification or by influencing chemical bioavailability. Furthermore, the ability of these fungi to form extended mycelial networks, the low specificity of their catabolic enzymes and their independence from using pollutants as a growth substrate makes them well suited for bioremediation processes (Harms *et al.* 2011).

POTENTIAL OF MUSHROOMS IN MYCORE-MEDIATION

Although bioremediation by bacterial agents has received the attention of workers, the role of fungi has been inadequately studied. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them for bioremediation. Mushroom-forming fungi (mostly basidiomycetes) are amongst nature's most powerful decomposers, secreting strong extracellular enzymes due to their aggressive



Fig.2: Commonly occuring white-rot fungi involved in mycoremediation. (a) Genus Agaricus; (b) Genus Agrocybe; (c) Genus Auricularia; (d) Genus Clitocybe; (e) Genus Coprinus; (f) Genus Fistulina; (g) Genus Flammulina; (h) Genus Ganoderma; and (i) Genus Laetioporus.



Fig.3:Fungus-metal interaction.Metal mobilizationresults from the production and excretion of organic acids (for example, citrate and oxalate), which increase metalsolubility through acidification of the mycosphere and provision of metal-complexing structures. This frequently occurs as a side effect of the dissolution of primary minerals containing phosphate, carried out by mycorrhizal fungi. Siderophores are chelators excreted for the acquisition of iron, and they may cross-react with other metals. Extra-hyphal immobilization occurs through the formation of secondary minerals, biosorption to cell wall constituents such as chitin and chitosan, complexation by glomalin (that is, metal-sorbing glycoproteins excreted by arbuscularmycorrhizal fungi) and effects of fungal mycelia and glomalin on soil aggregate stability against wind and water erosion. Metal uptake occurs, for example, through specific transporters for the acquisition of essential metals, and these transporters may cross-react with other metals. Intracellular metal immobilization involves storage in vacuoles and complexation by cytoplasmic metallothioneins144 and phytochelatins (that is, proteins and peptides, respectively, that are rich in SH groups). Metal transformations such as reactions involving organometals (for example, methylations) and redox reactions frequently result in metal volatilization. Streams of cytoplasmic vesicles and vacuoles along fungal hyphae may translocate metals to other parts of the mycelium and to the plant symbionts of the fungi. MnP, manganese peroxidase.

growth and biomass production. These enzymes include lignin peroxidases (LiP)(Ruiz-Duenas *et al.* 2008; Hofrichter *et al.* 2010), manganese peroxidase (MnP) (Ruiz-Duenas *et al.* 2008) and laccase (Majeau *et al.* 2010), etc. Thus, carbon sources such as sawdust, straw and corn cob can be used to enhance the degradation rates by these organisms at polluted sites (Adenipekun and Lawal, 2012). In short, white-rot fungus (Fig 2; Table 2) accounts for at least 30% of the total research on fungi that are used in bioremediation.

ADVANTAGES OF MYCOREMEDIATION

Mycoremediation technologies assist fungal growth and increase its population by creating optimum environmental conditions for them to detoxify the maximum amount of contaminants. A fungus produces various enzymes which are nonspecific, means that they can act on various environmental pollutants. There are numerous advantages of using mycoremediation over commercialized technologies, including the following:

(1)It is a natural system and does not introduce any corrosives or harmful chemicals for cleanup;(2)The process is environmentally friendly and works on a variety of organic and inorganic compounds; (3) Mycoremediation is expected to be safer than most other alternatives of bioremediation. It does not require digging up contaminated products and disposing of it at waste sites; (4)The technology is simple than many other alternatives; (5)Low maintenance and reusable of end products ; (6) The cost of using mycoremediation is relatively low in comparison to other technologies and treatment methods, as it does not require the building of new structures and (7) The technology shows immediate results. There is immediate mitigation of odor and visible improvement to a site. For end results, mycoremediation is quicker than other technologies, such as phytoremediation and bacterial bioremediation.

CONSTRAINTS FOR MYCOREMEDIATION

The use of higher fungi like mushrooms has been known in the remediation of polluted soil for some years only. Research has shown that mushroom species like *P. ostreatus* and *P. chrysosoporium* have emerged as model systems for studying bioremediation. But, a great deal still remains to be learned about the basic knowledge of how this white-rot fungus removes pollutants. Mycoremediation is a very important process but still, there are various problems that are hindering the potential of mycoremediation (Thakur, 2014).

FUTURE PROSPECTS AND CHALLENGES

Recent advancements - the addition of required potential fungal strains to the soil and the enhancement of the indigenous microbial population and its ability to break down various contaminants have proven successful. Whether the fungal mycelia are native or newly introduced to the site, the process of destroying contaminants is important and critical for understanding mycoremediation. Further, the application of this technology in large scale projects will demand much more work to streamline the methodologies. Once the research and development get started, the technology must pass evaluations at the local, state and federal levels, which requires funding and also the time to do so. With appropriate funding, certain products could be developed and made available for licensing and commercialization. However, current funding has been limited. But extensive research needs to be pursued as the technology has proven successful. Researchers feel that this technology is expected to be faster and more cost effective than other remediation technologies once it is commercialized. The use of fungi for remediation would allow commercial concern to offer inexpensive,

safe products to their customers. If the underexploited potential of fungus mycelium is further exploited, it will go a long way in eradicating pollution from soil (Thakur, 2014).

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