

Mycoremediation - a potential tool to control soil pollution

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SUMMARY : One of the major environmental problems faced by the world 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. Examples of fungi used as mycoremediators are - *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Phanerochaete chrysosporium*, *P. sordida* and *Trametes hirsute*, *T. versicolor*, *Lentinus edodes* and *L. tigrinus*. Mycoremediation application falls into different categories. *In situ* mycoremediation treats the contaminated soil in the location in which it was found, whereas, *ex situ* processes require excavation of contaminated soil before they can be treated. However, despite being the living dominating biomass in 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. The present review aims to promote the potential of fungi as mycoremediators to remediate soil pollution.

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Soil pollution has significant deleterious consequences for 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 (Watanabe, 2001). 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. Watanabe

(2001) 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 (Asgher *et al.*, 2008; Haritash and Kaushik, 2009). During 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 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 (Singh, 2006).

Mycoremediation is one of the most complex and technical areas of bioremediation, which refers specifically to the use of fungal mycelium. It is a process of using fungi to degrade or sequester contaminants in the

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contaminated environment. Jagtap *et al.* (2003) discussed mycoremediation 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. 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 biodegraders and the resultant compost has been used to enhance the growth of plants as well as bioremediation activity in the environment (Jagtap *et al.*, 2003). Mycelia of fungi are unique among microorganism having the ability to enhance plant growth. They secrete variety of extracellular enzymes involved in pollutants degradation. Some fungi are hyperaccumulators and are capable of absorbing and concentrating heavy metals in the fruiting bodies of mushrooms (Jagtap *et al.*, 2003). 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 (Bennet *et al.*, 2001). They reported that bioremediation technology leads to degradation of pollutants and may be a lucrative and environmentally beneficial alternative.

In present scenario soil pollution is increasing day by day. The content of toxic and heavy metals in the environment is increasing day by day and is emerging as a serious problem. Many hazardous waste sites require clean up, as they may include salt, organic heavy metals, trace elements, radioactive components etc. Various methods like, thermal, chemical and other physical treatment methods have failed to eliminate the pollution problem because those methods can only shift the pollution to a new phase such as air pollution (Williams *et al.*, 1992). The simultaneous clean up of all these contaminants by thermal / chemical / conventional method is technically difficult and expensive and these very methods can also destroy other soil biotic components (Dua *et al.*, 2006). Therefore, mycoremediation is an emerging clean up technology for polluted sites. The present review, therefore, aims to study the role of fungi and exploit their potential to remediate polluted soil.

Role of fungi in mycoremediation:

Fungi have been harnessed in many diverse applications since 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 (D'Annibile *et al.*, 2006). An edible and medicinal fungus also plays an important role

as natural environment remediator (Pletsch *et al.*, 1999; Matsubara *et al.*, 2004). The pivotal role of microorganisms and their limitations for mycoremediation must be understood in order to utilize them more efficiently. 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 ecosystem and may be a clean, simple and relatively inexpensive method of environmental remediation, especially if species native to each site are 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).

Loske *et al.* (1990) reported the main contaminants of polluted soils as follows:

Polycyclic aromatic hydrocarbons (PAH's):

Polycyclic aromatic hydrocarbons (PAHs) are the class of hydrocarbons containing two or more fused aromatic hydrocarbons. These are residues from the processing of oil, tar, coal and comparable substances.

Polychlorinated biphenyls (PCB's):

These are used as cooling agents in transformers.

Dioxines:

These are by-products of chemical manufacturing and are found in fly-ashes from combustion processes.

Alexander (1994) reported that the ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation. Fungi are among nature's most powerful decomposers, secreting strong enzymes. The great potential of fungi in bioremediation is by virtue of their aggressive growth, great biomass production and extensive hyphae reach in the environment (Ashoka *et al.*, 2002).

Mycoremediation a powerful tool:

Fungal mycelia have been considered to be primary governors for maintaining ecological equilibrium because they control the flow of nutrients. The strength and health of any ecosystem is a direct measure of its main divers – the fungal populations and their interaction with other organisms such as plants, animals and bacteria. Using fungi as the starter culture species in a mycoremediation project sets the stage for other organisms to participate in the rehabilitation process. The introduction of fungal mycelium into a polluted

site triggers a flow of activity and begins to replenish the polluted ecosystem.

Mycoremediation is an economically and environmentally sound alternative for bioremediation. It restores the value of depleted and polluted land. Currently, burning, hauling have been the common practices to remove / clean up the toxic waste. But because of these processes, environments do not get rid of all the waste materials and cannot restore the ecology. On the other side, this leaves the soil lifeless and contaminated. Various toxins (including mercury, PCB's, and dioxins etc.) are added to our food chain and become more concentrated at each and every step. Fungal mycelia can destroy these toxins in the soil before they enter our food supply chain. Mycoremediation is thus, a biological mechanism to destroy, transform or immobilize environmental contaminants (Adenipekun and Lawal, 2012).

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 (Hauke *et al.*, 2011).

Potential of mushrooms in mycoremediation:

Although, bioremediation by bacterial agents has received 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 extra cellular enzymes due to their aggressive growth and biomass production (Elekes

and Busuioic, 2010). These enzymes include lignin peroxidases (LiP), manganese peroxidase (MnP) and laccase, 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). *Phanerochaete chrysosporium*, *Agaricus bisporus*, *Trametes versicolor* and *Pleurotus ostreatus* amongst many mushrooms have been reported in the decontamination of polluted sites (Adenipekun and Lawal, 2012). Mushrooms have long been known for their nutritive and medicinal benefits. Sesli and Tuzen (1999) reported that mushrooms can be used to evaluate the level of environmental pollution and to remediate the metal polluted soil. Also, many studies have been carried out to evaluate the possible danger to human health from the ingestion of mushrooms containing heavy metals (Tismal *et al.*, 2010; Ouzouni *et al.*, 2009; Sesli and Tuzen, 1999).

Based on literature and studies, white rot fungus accounts for at least 30 per cent of the total research on fungi used in bioremediation (Adenipekun and Lawal, 2012). White rot fungi have been used for bioremediation of pesticides, degradation of petroleum hydrocarbons and lignocellulolytic wastes in the pulp and paper industry. White rot fungi are excellent mycoremediators of toxins held together by hydrogen-carbon bonds. Enzymes secreted by white rotters include lignin peroxidases, manganese peroxidases and laccases. Some specific examples of macrofungi mycelium especially white rot fungus used for mycoremediation are:

Phanerochaete chrysosporium:

Among the fungal species, *Phanerochaete chrysosporium* is emerging as the model system for bioremediation. *P. chrysosporium* has been known to degrade many types of organopollutants such as polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls and dioxines, chlorophenols, chlorolignins, nitrocranditics, synthetic dyes and different pesticides (Leonardi *et al.*, 2007; Adenipekun and Lawal, 2012). Sasek and Cajthaml (2005) examined several powerful degraders for example, *Phanerochaete sordida* (P. Karst. Y). Erikss, *Pleurotus ostreatus* (Jacq. Fr.) P. Kumm, *Trametes versicolor* (L. Fr.) Lloyd, *Nematolana frowardii* (Speg.) e. Herak and *Irpex lacteus* (Fr.) Fr. *P. chrysosporium* has been shown to affect the bioleaching of organic dyes (Nigam *et al.*, 1995). The first extracellular enzyme (ligninase) discovered to depolymerize lignin and lignin sub-structured compounds *in vitro* were produced by this fungus (Aitken and Irvine, 1989). Bumpus *et al.* (1985) proposed the use of this fungus in mycoremediation. Barr and Aust (1994) reported that *P. chrysosporium* has been successful in degrading toxic substances such as aromatic hydrocarbons, chlorination organics, insecticides, pesticides, nitrogen aromatics and laccases.

Lentinus edodes:

Shiitake mushroom can degrade pentachlorophenol (PCP), a broad-spectrum biocide that is more toxic than DDT in the soil (Adenipekun and Lawal, 2012).

Lentinus tigrinus:

Chlorobenzoic acids (CBA) are ubiquitous organic contaminants with different degrees of chlorination in soil. The fungus, *L. tigrinus* can mycoremediate CBA from the contaminated soil (Stella *et al.*, 2012).

Lentinus squarrosulus (Mont.) Singer:

This mushroom has been found to mineralize soil contaminated with various concentrations of crude oil resulting in increased nutrient contents in treated soil. Adenipekun and Fasidi (2005) reported the ability of *L. squarrosulus* to mineralize soil contaminated with various concentrations of crude oil (1 to 40%). Adenipekun and Isikhuemhen (2008) and Clementina *et al.* (2008) investigated the bioremediation of engine oil polluted soil by *L. squarrosulus*.

Pleurotus ostreatus (Jacq. Fr.) P. Kumm:

Oyster mushrooms are very commonly used for mycoremediation. Recent studies have shown that *P. ostreatus* is able to degrade a variety of polycyclic aromatic hydrocarbons (PAH) (Sack and Gunthen, 1993). It has the ability to degrade PAH in non-sterile soil both in the presence and in the absence of cadmium and mercury. It has been reported to catalyze humification of anthracene, benzo (a) pyrene and flora in two PAH – contaminated soils from a manufactured gas facility and an abandoned electric cooping plant (Bojan *et al.*, 1999). The Pacific Northwest National Laboratory (PNNL) has found that particular strains of fungi may be used to remediate oils, petroleum products, pesticides, PCB's and other hydrocarbon based pollutants (Sykes, 2002). *P. ostreatus* substrate has been used to biotreat Nigerian oil-based drill cuttings containing polycyclic aromatic hydrocarbons (PAHs) under laboratory conditions (Okparanma *et al.*, 2011). *Pleurotus ostreatus* significantly can degrade the toxic chemicals with certain alterations in the biodegradation strategies and can be a potential candidate for the reclamation of sites polluted with polyaromatic hydrocarbon (Baldrian *et al.*, 2000; Eggen and Majcherczyk, 1998; Eggen and Sveum, 1999; Bhattacharya *et al.*, 2012).

Pleurotus tuber-regium (Fries) Singer:

The white-rot fungus, *P. tuber-regium* is another fungus which has been studied for its ability to ameliorate crude oil polluted soil. Isikhuemhen *et al.* (2003) reported that the fungus had the ability to remediate crude oil polluted soil

and the resulting soil sample supported seed germination and seedling growth of *Vigna unguiculata*. They reported a significant improvement in percentage germination, plant height and root elongation. Adenipekun *et al.* (2011b) studied bioremediation of contaminated soil by *P. tuber-regium* Singer and reported that there was an improvement in the nutrient status of the soil and an increase in enzyme activity.

Pleurotus pulmonarius:

Adenipekun *et al.* (2011a) also worked on the management of cement and battery polluted soils using *P. pulmonarius*. A general increase was observed in the carbon content, organic matter, phosphorus and potassium and a decrease in percentage nitrogen, calcium and pH after 10 weeks of the incubation of mycelium. The lead content was constant in both polluted soils while a significant decrease was observed in the copper, manganese and nickel contents of the soils.

Trametes versicolor:

Aged mycelium from oyster mushrooms (*Pleurotus ostreatus*) mixed in with 'compost' made from woodchips and yard waste (50:50 by volume) resulted in far better degradation of hydrocarbons than oyster mushroom mycelium or compost alone (Stametes, 2010). *Trametes versicolor* produced three lignolytic enzymes with efficient degradation capacity on lignin, polycyclic aromatic hydrocarbons, polychlorinated biphenyl mixture and a number of synthetic dyes (Tanaka *et al.*, 1999; Novotny *et al.*, 2004). It has also demonstrated the ability to degrade dieldrin in the lab (Morgan *et al.*, 1991; Gadd, 2001).

Bjerkandera adusta:

Ligninolytic fungus, *Bjerkandera adusta* has the capacity of PAH degradation. The ligninolytic enzymes involved in the degradation of PAHs are - lignin peroxidase, versatile peroxidase, Mn-peroxidase and laccase (Adenipekun and Lawal, 2012). Pozdnyakova (2012) studied the PAH degradation during mycoremediation of PAH-contaminated soils by this fungus.

Irpex lacteus:

Bhatt *et al.* (2002) demonstrated the mycoremediation of PAH contaminated soils. They studied the effects of *I. lacteus* and *P. ostreatus* in degrading 7 three and four ring PAHs in two contaminated soils. Adenipekun and Lawal (2012) also reviewed the role of this fungus in mycoremediation.

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 non-specific, means that they can act on various environment pollutants. Hyphae allow fungi to expand their surface area, make them easier to contact the pollutant. There are numerous advantages of using mycoremediation over commercialized technologies, including the following:

Public acceptance:

It is a natural system, and does not introduce any corrosives or harmful chemicals for cleanup. In most cases, researchers use only native species on every site. Mycoremediation basically reduces the amount of wastes to be land filled.

Natural and environment friendly:

The fungal mycelium corrects an imbalance due to a contamination event or situation, and restores the natural function and creates a balance in the system. The complete protocol is natural and helps in bringing the contaminants within levels, within the ecosystem that are no longer harmful. The process is environmental friendly and works on a variety of organic and inorganic compounds.

Safety:

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. Additionally, this process does not produce any kind of secondary metabolites that require additional cleanup after the initial remediation process.

Simple and quiet:

The technology is simple than many other alternatives. This has structures, no machinery involved, and no noise. The system takes a day to set up, much like a landscaping project and then left to do its work.

Low maintenance:

There is minimal handling and low maintenance of sites treated with fungal mycelium. On the other hand, bioremediation using bacteria and phytoremediation requires repeated application of the same on weekly or biweekly tilling and turning, a lot of labour and maintenance. Mycoremediation can be done either on-site or off-site and with very low setup and maintenance charges.

Reusable end products:

The end products of mycoremediation are always nontoxic. The enriched and cleaned soil can be used for landscaping, road underlayment, or other purposes also.

Low cost:

The cost of using mycoremediation is relatively low in comparison to other technologies and treatment methods, as it does not require building of new structures.

Flexibility:

Mycoremediation is a very flexible process and additionally, fungal treatments can work in almost any kind of habitat and season. The size of the application can vary without any problem, and can be the size of a tumbler to acres across.

Fast:

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. These treatments may require one to three years or more, and cannot address all the contaminants that fungus can attack. Fungal treatment requires weeks to months. This process is very easy to implement and maintain.

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. chrysosporium* 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. Major constraint of this process was *Phanerochaete chrysosporium* as the major work on mycoremediation is on this fungus only. The ability is generally attributed to lignin – degrading enzymes system of this fungus. A similar degrading ability was described by many other species of white rot fungus but of not that very success. Sasek (2003) reported that the performance of white rot fungus in soil bioremediation depends upon its survival in the soil environment, colonization, relationship and interaction with other soil microflora present in the soil. Most of the results have been obtained on single fungal species *i.e.* *Phanerochaete chrysosporium* only.

Mycoremediation is a very important process but still there are various problems that are hindering the potential of mycoremediation. Boopathy (2005) discussed some of the factors limiting bioremediation technologies. Various challenges faced are:

The challenges faced in the field application such as contamination by other fungi especially *Penicillium* spp., *Aspergillus* spp. needs to be researched to recommend solutions.

This fungus has the inability to compete with native microbes in soils. Bacteria could either inhibit the growth of

fungi or in combination with fungi, enhance degradation of pollutant.

The nutrient, a requirement of the fungus has to be completely understood so as to enable it to thrive at a contaminated site.

One problem to be borne in mind is that in bioremediation projects mushroom mycelium should not be used as a starter species.

There are also legal issues in this process. There are several patents specifically granted for matching fungus against a toxin. This is a major hindrance in preventing wide-scale fungal clean-up of toxins from the polluted site.

The lack of experienced mushroom cultivators in outdoor trials is a problem in mycoremediation. This lacking has affected the success of several trials.

Future aspects:

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. There is no definite time frame for complete mycoremediation as the time taken by various contaminants and types of applications will vary. That is why the research in this area is still in the experimental phase. Further, the application of this technology in large scale projects will demand much more work to streamline the methodologies. Once the research and development gets 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.

Conclusion:

Fungi can be used as a tool to reduce waste materials in contaminated soils via non-specific enzymes activity. The evidences have shown that mushrooms have the potential to clean up soil contaminated with different toxic elements. Mycoremediation is not a panacea, but an effective and powerful tool to remediate soil pollution. In fact it can play a

pivotal role in breaking down numerous toxic substances. Myco-facilitation can help to transform a degraded location into a thriving ecosystem with increased diversity. Therefore, mycoremediation needs well trained personnel with sufficient knowledge of microbiology. Both intrinsic (deeper study of the influence of mycoremediation process) and extrinsic (new possibilities for fungal species) research is needed to bring about the desired results. Despite the dominating living biomass in soil, fungi have not been fully exploited for the bioremediation of such polluted environments. In this review, the metabolic and ecological features that make fungi suited for use in mycoremediation are described and their potential for applications discussed. More extensive research needs to be carried out on the potential of mushrooms and fungal species for mycoremediation.

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