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Review

Uses of mushrooms in bioremediation: A review

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One of the major environmental problems facing the world today is the contamination of soil, water and air by toxic chemicals as a result of industrialization and extensive use of pesticides in agriculture. Incineration is currently the most effective and common remediation practice but is costly in terms of money and energy used. A rapid cost effective and ecologically responsible method of clean-up is "bioremediation" which utilizes micro-organisms to degrade toxic pollutants in an efficient economical approach. Toxic chemicals are degraded to less harmful forms. Although, bioremediation by bacteria 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. 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 degradation rates by these organisms at polluted sites. White rot fungi have been used for biotransformation of pesticides, degradation of petroleum hydrocarbons and lignocellulolytic wastes in the pulp and paper industry. *Phanerochaete chrysosporium*, *Agaricus bisporus*, *Trametes versicolor* and *Pleurotus ostreatus* amongst many mushrooms have been reported in the decontamination of polluted sites. In Nigeria, *Lentinus squarrosulus*, *Pleurotus tuber-regium*, *P. ostreatus* and *P. pulmonarius* have been employed in bioremediation of contaminated soils both *in-situ* and *ex-situ*. This paper highlights the use of fungal mycelia in bioremediation (myco-remediation) and studies on the uses of mushrooms for bioremediation.

Key words: Bioremediation, mushrooms, polluted soils.

INTRODUCTION

The pollution of the environment with synthetic organic compounds has become a major problem world wide. Many of these novel compounds introduced to the nature are called xenobiotics being materials that do not occur naturally in the biosphere and many of which are not easily degraded by the indigenous microflora and fauna (Sullia, 2004).

There are several classes of chemicals that have been targeted by United States Environmental Agency (USEPA) as priority pollutants due to their toxic effects on the environment and human health. These chemicals include polycyclic aromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, 1,1,1- trichloro – 2,2-bis (4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene and trinitrotoluene. Polycyclic aromatic hydrocarbons (PAH) are recalcitrant environmental contaminants that are generated from the burning of fossil fuels, coal mining, oil drilling and wood burning (Lau et al., 2003; Verdin et al., 2004).

Currently, incineration is the most effective and common remediation practice, but this is extremely costly, in terms of money and energy used. All of these chemical compounds pose a significant threat to the health and vitality of the earth system (Hamman, 2004). The elimination of wide ranges of pollutants and wastes from the environment is therefore an absolute requirement to promote a sustainable development of our society with low environmental impact. Due to the magnitude of this problem and the lack of a reasonable solution, a rapid cost-effective ecologically responsible method of clean-up is greatly needed (Hamman, 2004).

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According to Atlas and Bartha (1992), bioremediation is the onsite enhancement of live soil organisms such as fungi, bacteria and green plants to breakdown hydrocarbon and organic contaminants. It involves the application of organisms and nutrients to contaminated soil to enhance biodegradation. Micro-organisms used in bioremediation must have been tested and proven to be successful in laboratory studies. Bioremediation may be employed to attack specific soil contaminants such as degradation of chlorinated hydrocarbons by bacteria. A mere general approach is the cleanup of oil spills by the addition of nitrate and phosphate fertilizers to facilitate decomposition of crude oil by indigenous or exogenous bacteria.

Loske et al. (1990) reported that the main contaminants in polluted soils are:

1. Polycyclic Aromatic Hydrocarbons (PAH's) viz. residues from the processing of oil, tar, coal and comparable substances.
2. Polychlorinated Biphenyls (PCB's) used as cooling agents in transformers.
3. Dioxines: These are by-products of chemical manufacturing and are found in fly-ashes from combustion processes.

The general approaches to bioremediation are to enhance natural biodegradation by natural organisms (intrinsic bioaugmentation). Unlike conventional technologies, bioremediation can be carried out *in-situ* and *ex-situ*. *In-situ* bioremediation involves treating contaminated materials at the site while *ex-situ* involves the removal of the contaminated material to be treated elsewhere (http//en.wikipedia.org.wiki/bioremediation).

The literature concerning organic chemical wastes (xenobiotics) dealt almost exclusively with bacteria. Whenever bioremediation is discussed, bacteria agents come into focus and fungi are much less studied. It is now becoming apparent that fungi also play an important role in degrading organic materials in the ecosystem and that they have potential for remediating contaminated soils and water.

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).

Fungi require substrates such as cellulose or other carbon source as a source of energy. Thus, carbon sources such as corn cobs, straw and sawdust can be easily used to enhance degradation rates by these organisms at polluted sites. Also, the branching filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil (Hamman, 2004). Fungi are unique among microorganisms in that they secrete a variety of extracellular enzymes involved in pollutant degradation. They use a variety of mechanisms to accomplish the degradation of lignin and a wide variety of other environmental pollutants (Asamudo et al., 2005).

The easiest method of treating contaminated soil is to simply add organic matter to as many toxic metals will readily form compounds with the organic materials found in compost (Kellogg and Pettigrew, 2005). The concept of composting originally applicable to waste conversion into mulching and soil conditioner is now being applied to the hazardous waste treatment.

There is a lot of descriptive bioremediation concerning the ability of various fungi and their enzymes to biotransform pesticides (Raj et al., 1992). To date, the most sophisticated fungal approach to environmental clean-up has grown out of prior research on degradation of petroleum hydrocarbons (Atlas and Bartha, 1992; Cerniglia et al., 1992).

MUSHROOMS AND BIOREMEDIATION

White-rot fungi are so called because their degradation processes result in a bleaching of wood substrates (Kirk et al., 1992). They digest lignin in wood by the secretion of enzymes giving wood a bleached appearance.

The white-rot fungi technology is quite different from other well-established methods of bioremediation (for example, bacteria systems). The differences are primarily due to the unusual mechanisms which nature has provided them with several advantages for pollutant degradation (Asamudo et al., 2005).

One distinct advantage these fungi have over bacterial systems is that they do not require preconditioning to the particular pollutant. Bacteria usually must be pre-exposed to a pollutant to allow the enzymes that degrade the pollutant to be induced. The pollutant also must be in a significant concentration, otherwise induction of enzyme synthesis cannot occur. Thus, there is a finite level to which bacteria can degrade pollutants (Asamudo et al., 2005). Various strains of white-rot fungi capable of degrading aromatic compounds were reported by Barr and Aust (1994).

Lang et al. (1995) reported that lignin decomposing white-rot fungi show extraordinary abilities to transform recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAH's). They added that this unique capability may be used for decontamination of oil-polluted soils although lignocellulosic substrates must be supplied for the survival of fungal species in the soil. White-rot fungi have been used in bioremediation of polluted soils and accumulation of heavy metals. They have also been found to be involved in mineralization, bio-deterioration, biodegradation, transformation and co-metabolism (Bennet et al., 2002).

Isikhuemhen et al. (2003) reported that white-rot fungi are increasingly being investigated and used in bioremediation because of their ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants.

WHITE-ROT FUNGI DEGRADATION SYSTEM

The main mechanism of biodegradation employed by this group of fungi is the lignin degradation system of enzymes. Extra-cellular lignin modifying enzymes (LME's) have very low substrates-specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that is structurally similar to lignin (Mansur et al., 2003; Pointing, 2001). The major components of lignin degradation system include lignin-peroxidase (LiP), manganese peroxidase (MnP), H_2O_2 producing enzymes (Kirk and Farrell, 1987) and laccase, although not all lignolytic fungi show the three types of enzymatic activity.

It has been demonstrated that a lot of species belonging to the group of white-rot fungi are able to degrade lignin, which is a naturally occurring polymer (Hattaka, 1994). This capacity is assumed to result from the activities of extracellular oxidases and laccases (Glenn and Gold, 1983). These enzymes are non-specific; they oxidize a wide range of xenobiotics (Barr and Aust, 1994; Martens et al., 1996). White-rot fungi have been proposed for the biodegradation of polluted sites containing complex mixtures of PAH's such as occurring in creosote, coal tar and crude oil (Loske et al., 1990). Isikhuemhen et al. (2011) carried out a solid state fermentation (SSF) experiment with *Lentinus squarrosulus* (strain MBFBL 201) on cornstalks and evaluated lignocellulolytic enzymes activity. The results showed that *L. squarrosulus* was able to degrade cornstalks significantly after 30 days. Maximum lignocellulolytic enzyme activities were obtained on day 6 of cultivation and are a good producer of exopolysaccharides. The very fast good rate of *L. squarrosulus* makes it an ideal candidate for application in industrial pretreatment and biodelignification of lignocellulosic biomass.

POTENTIALS OF MUSHROOMS IN BIOREMEDIATION

Phanerochaete chrysosporium

Among the fungal systems, *Phanerochaete chrysosporium* is emerging as the model system for bioremediation. *P. chrysosporium has* been known *to* degrade besides lignin macro molecules, many types of organopollutants such as polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls and dioxines, chlorophenols, chlorolignins, nitrocranditics, synthetic dyes and different pesticides. 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. were selected for the study (Sasek and Cajthaml, 2005).

P. chrysosporium has been shown to affect the bioleaching of organic dyes (Nigam et al., 1995). The first extracellular enzyme (ligninase) discovered to deploymerize lignin, and lignin sub-structured compounds *in vitro* were produced by this organism (Aitken and Irvine, 1989). *P. chrysosporum* has degraded toxic xenobiotics such as aromatic hydrocarbons, chlorination organics, insecticides, pesticides, nitrogen aromatics and laccases; polyphenol oxidases and lignin peroxidases being involved in the degradation process (Barr and Aust, 1994).

Phanerochaete flavido-alba

Phanerochaete flavido-alba has been able to decolorize Olive oil mill wastewater (OMW), a major waste product of olive oil extraction for subsequent use in bioremediation assays. Of several media tested, nitrogen-limited *P. flavido-alba* cultures containing 40 mg/L Mn (II) were the most efficient at decolorizing OMW. Decolorization was accompanied by a 90% decrease in the OMW phenolic content. Concentrated extracellular fluids alone (showing manganese peroxidase, but not lignin peroxidase activity) did not decolorize the major OMW pigment, suggesting that mycelium binding forms part of the decolorization process.

Trametes versicolor

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). *T. versicolor* and its enzymes have been reported to delignify and to bleach kraft pulp (Gamelas et al., 2005) and also efficiently dechlorinate and decolorize bleach kraft pulp effluents (Selvam et al., 2002). This presents a good potential to be the base of new environmental friendly technologies for pulp and paper industry. Amaral et al. (2004) also reported the use of *T. versicolor* as biocatalysts for decolorization of different industrial dyes and waste water treatments.

Pleurotus ostreatus (Jacq. Fr.) P. Kumm

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).

Pleurotus tuber-regium (Fries) Singer

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

In another investigation, observed an increase in nutrient contents (organic matter, carbon, available potassium) in soil polluted with 1 to 40% engine oil concentration after six months of incubation with *P. tuber-regium*. The fungus also brought about an increase in copper content in engine oil polluted soils at 10% concentration followed by a decrease at 20 and 40% concentrations. Bioaccumulation of zinc and nickel was recorded at 20% enzyme oil concentration.

Ogbo et al. (2006) investigated the effect of different levels of spent lubricating oil on the growth of *P. tuber-regium* at different levels (5, 8, 16, 30, 65, 98, 130 and 160%). The fungus grew optimally at 98% level of contamination. Average yield showed that 98% level of contamination gave the highest yield (79.56 g), although inhibition was noticed at 130 and 160%. The shortest and tallest sporophores were recorded in control and 5% level of contamination.

Ogbo and Okhuoya (2009) investigated the effect of crude oil on the yield and chemical composition of *P. tuber-regium* (Fr.) Singer on soils to which sawdust, shredded banana leaves, NPK fertilizer and poultry litter were added. The study showed that crude oil contamination improves the overall well being of the fungus. Substrate composition affects the nutrient status of the mushroom causing variation in the carbohydrate, protein, fat, crude fibre and ash content. Sawdust and poultry litter enhances crude oil fertilizer effect on the fungus while shredded banana leaf blades and NPK fertilizer reduces the fertilizer effect. The quality of the mushroom fruit body from the crude oil contaminated site was comparable to that cultivated under the best substrate composition for the mushroom".

Bioremediation of cutting fluids contaminated soil by *P. tuber-regium* Singer by Adenipekun et al. (2011a) reported that there was an improvement in the nutrient status of the soil and an increase in enzyme activity. A reduction in the pH and heavy metal contents of the soil at the levels of cutting fluids concentrations was detected. The lignin in the rice straw decreased from 34.50% in the control to 8.06 at 30% cutting fluids concentration after 3 months of incubation. The highest TPH loss of 30.84% was recorded at 20% cutting fluids contamination after 3 months compared to 13.75% at the onset of the experiment. The improvement of the nutrients contents of the soil, bioaccumulation of heavy metals, degradation of TPH, lignin, and increased activity of polyphenol oxidase and peroxidase was due to biodegradation of the cutting fluids.

Lentinus squarrosulus (Mont.) Singer

Lentinus squarrosulus 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%). They found that nutrient contents were generally higher after 6 months of incubation except potassium levels which were not increased. The highest rate of biodegradation was at 20% concentration after 3 months and 40% after 6 months of incubation (Adenipekun and Fasidi, 2005).

Adenipekun and Isikhuemhen (2008) investigated the bioremediation of engine oil polluted soil by *L. squarrosulus*. Results indicated that contaminated soils had increased organic matter, carbon, available phosphorus while nitrogen and available potassium reduced. A relative high percentage degradation of total petroleum hydrocarbon (TPH) was observed at 1% engine oil concentration (94.46%) which decreased to 64.05% TPH degradation at 40% engine oil concentration after 90 days of incubation. The concentrations of Fe, Cu, Zn and Ni recovered from the fungal biomass complex increased with increase of engine oil contamination. The improvement of nutrient content values as well as the bioaccumulation of heavy metals at all levels of engine oil concentrations tested through inoculation with *L. squarrosulus* is of importance for the bioremediation of engine polluted soils.

Isikhuemhen et al. (2010) carried out the preliminary studies on Mating and Improved Strain Selection in the Tropical Culinary-Medicinal Mushroom *L. squarrosulus* Mont. (Agaricomycetideae). The rapid mycelia growth and enhanced enzyme production by *L. squarrosulus* have biotechnological applications for wood and pulp, textile, and tanning, as well as in the bioremediation of oil spills. Furthermore, the results showed that intrastock mating resulted in some dikaryons that outperform their parent strains in growth rate and speed to primordial initiation. This reinforces the importance of breeding and selection during the domestication of wild edible and medicinal fungi of interest.

Pleurotus pulmonarius

Adenipekun et al. (2011b) also worked on the management of cement and battery polluted soils using

Pleurotus 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 incubation. 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. The polyaromatic hydrocarbons (PAH) content also decreased from 6.86 in control to 0.56 after 10 weeks of incubation.

OTHER WHITE-ROT FUNGI

The vegetative growth responses of three local edible mushrooms, *P. pulmonarius, L. squarrosulus* and *P. tuber-regium* on different concentrations of crude oil, automotive gasoline oil (AGO), fresh engine oil and spent engine oil were investigated. *P. tuber-regium* grew fastest among the three organisms on all pollutants and radial growth was observed at all concentrations. Almost the same pattern of growth was observed for *P. pulmonarius* and *L. squarrosulus*. Radial growth for both was supported by crude oil and AGO at all concentrations whereas growth on engine oil (fresh and spent) was not observed beyond 10% concentration. The ability of these mushrooms to tolerate the pollutants and grow on them suggests they could be employed as bioremediation agents on sites contaminated by these pollutants (Adedokun and Ataga, 2006).

Olive oil mill wastewater (OMWW) was used as a substrate for the culture of a mixture of edible fungi in order to obtain a potentially useful microbial biomass and to induce a partial bioremediation of this fastidious waste (Laconi et al., 2007). The fungal mixture grew fairly well in the treated OMWW and reached a maximum of biomass production within about 14 days of fermentation at room temperature. Up to 150 to 160 g of wet biomass was obtained per liter of OMWW. Analysis of the partially dehydrated biomass revealed a protein content of about 13 and 6 g% of row fiber. A relevant presence of unsaturated fatty acids was found, as well as the presence of significant amounts of vitamins A and E, nicotinic acid, calcium, potassium and iron in the *Pleurotus* species.

Emuh (2010) reported that the crude oil and heavy metals present in polluted soil are broken down and absorbed by mushroom hypha and mycelia through the secretion of enzymes into environmentally safe levels resulting in carbon (IV) oxide, water and perhaps biomass. Zebulun et al. (2011) worked on the decontamination of anthracene-polluted soil through a white rot fungus (*P. ostreatus*) induced biodegradation. They reported that time, level of contamination and fungal treatment affected the rate of degradation of all levels of anthracene degradation (76 to 89%) compared to control soil (33 to 51%). It was reported that the release of lignolytic enzymes such as lignin peroxidase, laccase and manganese peroxidase by *P. ostreatus* are associated with the degradation of anthracene. Increase in the concentration of anthracene at different sampling dates in some of the soil samples was also observed.

Mycoremediation (fungal treatment or fungal-based technology) is the application of fungi in the remediation of polluted soil and aqueous effluents. It is an aspect of bioremediation which is defined as the biological process that involves the filtration of agricultural/industrial water runoff, decomposition of hydrocarbon based contaminants, concentration/removal of heavy metal from soil and other substrates by a biological organism (Stamets, 1999).

Mycoremediation of crude oil and palm kernel contaminated soils by *P. pulmonarius* was tested by Adenipekun and Lawal (2011). They reported an increase in the nutrient contents of palm kernel sludge contaminated soil. A decrease in the heavy metal contents was observed at all level of crude oil except lead Pb which increased at 5 and 20% crude oil contamination. An increase was observed at all levels of palm kernel sludge contamination except Zn which decreased from 2.97 to 2.75 mg/kg, Lead Pb also decreased at 2.5, 20 and 40%, and Cu at 1 and 40% in palm kernel sludge contamination after 2 months of incubation. The Total Petroleum Hydrocarbon showed a percentage loss of 40.80% at 1% crude oil concentration and 9.28% at 40% crude oil contaminated soil after 2 months. The lignin content of the rice straw reduced from 13.56% in the control to 7.71% and organic matter content decreased from 37.96 to 17.90% after 2 months. The improvement of nutrient content value as well as the bioaccumulation of heavy metals at all levels of crude oil concentrations tested through inoculations with *P. pulmonarius* is of importance for the myco-remediation of crude oil and palm kernel sludge polluted soil.

Okparanma et al. (2011) reported that spent white-rot fungi (*P. ostreatus*) substrate can be used to remediate Nigerian oil-based drill cuttings containing poly-aromatic hydrocarbons and also serve the dual purpose of reducing the bulk volume of the spent fungal substrate as a waste and the incidence of occurrence of PAH's in the environment. After 56 days of composting, the total amount of residual PAH's in the residual cuttings decreased from 19.75 to 7.62%, while the overall degradation of PAH's increased to between 80.25 and 92.38% with increasing substrate addition.

The use of higher fungi like mushrooms has been known in the remediation of polluted soil for some years now. Research has shown that mushroom species like *P. ostreatus* and *P. chrysosoporium* have emerged as model systems for studying xenobiotics degradation. A great deal still remains to be learned about the fundamentals of how this white-rot fungus mineralizes pollutants; not surprisingly, even less is known about the degradative mechanisms used by fungi in general. Though oxidative enzymes play a major role, organic acids and chelators excreted by the mushroom also contribute to the process and these still need to be studied. Also, most of the work on mycoremediation that has been carried out in Nigeria especially is under laboratory conditions. It is recommended that *in situ* applications of white-rot fungus on polluted soil should be carried out especially in the Niger Delta area of Nigeria where the problem of oil spillage is a daily occurrence (Fasidi et al., 2008).

CONCLUSION

The application of white-rot fungi in bioremediation is expected to be relatively economical because the fungi can be grown on a number of inexpensive agricultural or forest wastes such as rice straw, corn cobs and sawdust. The fungal inoculum can also be mass-produced by current simple techniques used to produce fungal spawn.

In the quest, for economical and ecologically sound methods for environmental remediation, the use of mushrooms is a very good approach and solution. More intensive research needs to be carried out on the potentials of bioremediation and ecology of a large number of edible mushrooms effective for bioremediation. The challenges faced in the field application such as contamination by other fungi especially *Penicillium* sp*.,* *Aspergillus* spp*.* needs to be also looked into and solutions recommended.

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