Bioremediation of Contaminated Riparian Zones Using Mycorrhizal Fungi – An Exploration of the Feasibility of Restoration Through Mycoremediation

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Abstract

Bioremediation of Contaminated Riparian Zones Using Mycorrhizal Fungi – An Exploration of the Feasibility of Restoration Through Mycoremediation

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The author uses an extensive literature review to explore the question "Does current scientific research support the use of higher order mycorrhizal fungi as a bioremediation tool in the restoration of riparian zones that have been altered or damaged by anthropocentric presence and activities?" Emerging scientific literature suggests that many native fungal species are able to alter or detoxify specific toxins like heavy metals, petroleum by-products and industrial effluents. Laboratory and field studies indicate the mycelial networks of these life forms may alter the chemical and molecular structures of the toxins, rendering them harmless, and reducing their negative impacts on the environment. The author concludes there is ample data in the research to suggest that mycorrhizal bioremediation techniques show considerable promise as biomediation factors in providing and recovering safe and healthy spawning and habitat areas for all riparian life forms, including fish species, and may also provide a method for reducing the presence of harmful substances in the food web for the entire planet.

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Gary K. Jones

Introduction

Does current scientific research support the use of higher order mycorrhizal fungi in the restoration of riparian zones that have been altered or damaged by anthropocentric presence and activities? There is emerging literature in the scientific media suggesting that many native fungal species are able to alter or detoxify specific environmental toxins and other environmental contaminants including heavy metals, pesticides, effluents, human hair, transmission lubricants, and certain grades of petroleum, like those spilled in the Exxon-Valdez disaster (Singh, 2006). Laboratory and field studies indicate the mycelial networks of these life forms may alter the molecular composition and structures of soil contaminants and other energy/nutrient sources during their metabolic breakdown, rendering the toxins either neutral or providing a convenient means to facilitate their extraction from the affected areas (i.e. harvesting or continued biodegradation) at reasonable levels of fiscal and natural economy and with minimal secondary impacts on the environment. This thesis examines the emerging scientific literature surrounding the feasibility of using fungal organisms as an applied technology for mediating toxic waste sites, not as a single solution, but as part of a well engineered and documented bioremediation strategy; the thesis focuses on how several projects can provide feedback for research and connect theory and research with practice through application in various treatment projects.

In some cases (Atlas et al, 2008) the introduction of the new fungal species may actually improve the bio-capabilities of the impacted area by improving soil conditions, returning the toxic molecules to the carbon cycle/web of life in newly transformed and benign structural compositions and encouraging the re-establishment of native plant and animal species. In this report the authors define bioremediation as "…the use of living organisms, especially microorganisms, to degrade pollutants and restore environmental quality." The authors go on to critically assess current contamination problems that can be treated by processes based on native microorganism introduction. Although most public information on aquatic oil spills center around images of dead birds and blackened shorelines, in fact most environmental contaminants come from much smaller hydrocarbon leakages, like waste motor oils and underground storage tanks (Atlas et al, 2008). Most of these spills or inputs are readily managed or accommodated "due to the capacities of microorganisms to biodegrade hydrocarbons." A common method for applying this technology, the report asserts, is to "…increase the natural rates of hydrocarbon biodegradation that produce nontoxic end products. These treatments may include adding microorganisms with specific enzymatic abilities to a contaminated site and establishing conditions that favor microbial degradative activities. In many cases, bioremediation can be carried out in situ. In other cases, contaminated soils and sediments must be treated in bioreactors to remove problem pollutants." Added seed cultures may or may not improve the biodegradation improvement rates for a given ecological system, as so many contributing factors affect the metabolic processes. Hydrocarbon degrading fungi and bacteria tend to be widely distributed, and therefore, the report suggests, the addition of engineered or processed substrates, inoculates, or mulches may not contribute much to natural processes already underway. It would seem, the authors continue, that the deposition of starter materials would be most useful in areas where the active biota systems have been radically altered, like logging roads, where the sub soils have been eroded away to the mineral layers, and in this case, straw mulches would definitely assist in building up organic matter and assist in developing conditions where life can get a fresh start. Prince William Sound is cited in this report as an example of how these local variants can impact the natural processes: within this marine area, the compounds pristine and phytane were rapidly degraded and served as a suitable internal reference marker for just a few weeks to months. Scientists theorize that this rapid degradation is due to the fact that microorganisms in Prince William Sound have evolved to effectively consume terpenes. Pine trees have been dripping terpenes into the sea for millions of years. Terpenes, which give the pine its distinctive odor, are isoprenoid hydrocarbons that have similar chemical structure to pristine and phytane. So, the Prince William Sound hydrocarbon degraders are very good at consuming these compounds.

Kirk, et al (1991), conducted a survey of several species of authochtonous marine, filamentous Ascomycetes and Deuteromycetes from southeastern Virgina, species that have been shown to use specific compounds as sole carbon sources for growth and to mineralize soils. The intent was to define a field characterization of fungal species that would effectively degrade petroleum byproducts and compounds in a variety of marine and near shore environments that were impacted directly and indirectly by the presence of marinas for pleasure and working craft aquatic vessels. The authors conclude that a need exists for additional research on the effectiveness of higher order aquatic fungal species as hydrocarbon degraders: "To date there are no field or microcosm studies to confirm whether arenicolous or other higher marine fungi degrade hydrocarbons in their natural environments, or have potential as bioremediation agents for marine petroleum spills (Kirk et al, 1991).

A second body of literature related to toxicology suggests that native fish species and higher order predators like bears and human beings, are negatively impacted by industrial effluents and other toxins that make their way into riparian zones that are critical in the life cycles of Pacific salmon and other species (Washington State Pesticide Monitoring Program Reconnaissance Sampling of Fish Tissue and Sediments, Washington State Department of Ecology, 1994). This paper will not specifically address the connections or processes that involve bioaccumulation and the impacts on salmon fry and migrating adults, but these are general areas of environmental concern that may benefit from work in the area of mycoremediation and the clean up of environmental toxins.

Most of us think of mushrooms as a sort of strange, fungal life form that appears in the forest or other dark, damp places following extended periods of rain. We associate them with fairies, toads, and poisonous concoctions like witches brew. We are happily unaware that the true vegetative body or sustaining structure of the organism is an invisible, subterranean network of biological cells called mycelia or mycelium (Singh, 2006). Interlacing membranes of mycelia form a mosaic of communicating, reactive cells that interact with and respond to changes in their environments, often with significant impacts. They employ a variety of enzymatic and chemical responses to complex changes in their habitat and the terrestrial strata of the earth. Mycelial networks that support the fruiting mushroom bodies extend from subsoil locations to rain forest canopies. Scientists started using bacteria and fungi to degrade xenobiotic organic compounds in the mid-twentieth century. Early successes in research favored bacterial applications until more recently, when research with so-called "white rot" fungi began to show more favorable results and increased activity in the area of fungal applications (Aust 1995, Singh 2006).

In his report to the Conference on Biodegradation: Its Role in Reducing Toxicity and Exposure to Environmental Contaminants (Aust, 1995), the author covers in detail a variety of mechanisms employed by white rot fungi to complete the degradation of lignin and a number of environmental pollutants: "The fungi secrete a family of peroxidases to catalyze both direct and indirect oxidation of chemicals. The peroxidases can also catalyze reductions using electron donors to generate reductive radicals. A cell-surface membrane potential can also be used to reduce chemicals such as TNT." White rot fungi are well known for their ability to biodegrade lignin in wood. They also mineralize (oxidize to CO2) some chemicals that are already highly oxidized. There is some debate over whether the peroxidases based system of the fungi is sufficient to completely degrade lignin, and that more research is needed to develop a full understanding of the processes involved. Several mechanisms, including membrane potentials, are discussed in an attempt to explain the uniquely effective degradative and reduction properties associated with the presence of white rot fungal species in soils and woody debris sites. Aust's report adds to a wide body of literature that supports the potential for applied uses of specific fungal species in the treatment of toxic sites in most terrestrial and near shore environments where these species are able to thrive and gain an ecological niche as part of a broader bioremediation strategy. There is no hint of a suggestion that the white rot fungus is a cure all or magic bullet for applied science or technologies.

Not all scientific evidence points to the same result, and any application of fungal species that is considered for real world situations must be carefully understood, and species selection must consider the target result that planners expect to achieve. The following study suggests that under certain conditions, the presence of mycorrhizal fungi in the soil or substrate may serve in a detrimental fashion by facilitating the uptake of heavy metals by plants that are infected by the fungi (D'Annibale et al, 2006).

The authors were particularly concerned with providing data that would help understand what happens to environments that receive widespread exposure to contaminants in so called “down wind” conditions – where a manufacturing process distributes toxins more or less randomly into surface soils, riparian zones, and other creature habitats. Some good examples are the Hanford Nuclear Site in south central Washington State, the ASARCO superfund smelter site on Commencement Bay in the same state, and Bhopal India, where eleven acres of highly contaminated material sits uncontrolled and aging in tin shacks following the release of phosphocyanic gas that directly killed thousands of people. Twenty – four years following the event there has been no direct action to assess the impacts of the disaster, or follow up on secondary effects on the health of the local population, or clean-up of the site.

Recent interest in the use of mycorrhizal enhancements that may be obtained through mycoremediation techniques has generated several productive and long needed studies that examine the exact mechanisms that are at work and how they might best be employed by scientists and soils/wetland engineers. Killham and Firestone (1983) investigated the hypothesis that since vesicular mycorrhizal fungi can stimulate plant metal uptake in soils where the metals are barely present, they might prove useful in reducing the presence of metals through uptake in cases where the host plants are growing in soils containing potentially toxic levels of heavy metals. The authors’ intent was to show the influence of such fungal infections on heavy metal uptake and growth of a perennial grass in a soil exposed to acidic and heavy metal depositions. This report generated the following conclusions: The influence of mycorrhizal fungal species infection can significantly impact the uptake of heavy metals under conditions of high relative acidity, as is commonly found in smelter effluent conditions. Several factors that play a role in this process are the specific condition and composition of the soil, acidity and metal content. Bioavailability of pollutant heavy metals was found to be more prevalent in sandy soils containing minimal amounts of clay and organic components. The authors go on to suggest that the processes which result in increased metal availability, like acidification, can also be expected to produce less buffering in these soil types. This study identifies potentially adverse consequences of fungal infection, and calls out the need for stringent guidelines and more outcome-specific type research that will enable scientists to apply the right tool to the job. Under conditions of acidic and heavy metal deposition, mycorrhizal fungal infections can greatly enhance metal uptake and result in reduced plant growth. This may or may not be a desired outcome, and should be factored into any planning effort involving these or similar sets of conditions.

Current Ecological Concerns

In a recent report on the effects of pesticides on Pacific Salmon (Ewing 1999), provides us with the results of a recent review of scientific literature on the effects of sublethal concentrations of pesticides on salmonids. The report emphasizes how pesticides can alter the biology of fishes in subtle ways that negatively impact their chances for survival and reproduction. Pesticides are only one in a variety of classes of toxins, including heavy metals and pharmaceutical by-products that move into streams and rivers throughout watersheds and have the potential for posing problems far from the site of application. One significant problem is that these chemicals do not necessarily disappear over time. They are transformed into other compounds that may be less toxic, equally toxic, or more toxic than the original material. Fish and other biological organisms must suffer the impacts of these toxins and cope as best they can with the results of their presence, which may be renewed and increased by repeated, uncontrolled, applications.

Through a process known as bioaccumulation, the concentration of these toxins in plant and animal tissues may be several times higher than that contained in the surrounding water or soils. This presents a significant environmental health risk to subsistence communities whose members rely on these food sources for survival throughout the year.

One well documented case (Ewing 1999) involved the release of the herbicide acrolein, which subsequently was determined responsible for the deaths of approximately 92,000 steelhead, 114 juvenile Coho Salmon, 19 resident Rainbow Trout, and thousands of non-game fish in Bear Creek, Oregon, a tributary of the Rogue River. Ewing's report describes the results of studies which show that, in contrast to immediate kill effects, many toxins have longer term behavioral impacts on salmon. These effects include stress in juveniles which may make them more susceptible to predation, effective swimming abilities which may reduce the ability to feed, avoid predation, defend territories, and maintain position in the river system. Many pesticides disrupt schooling behavior, a common observation by scientists, and the ability to seek optimal water temperatures during their upstream and downstream migrations.

Adult salmon are also known to adjust their migration patterns to avoid areas of pollution, which may delay spawning, create additional stress in the animal, and increase the energy consumption needs of the fish. Ewing (1999) makes a final observation that immune systems of salmon may also be disrupted as the result of exposure to toxic materials. One of the best documented cases of the immediate lethal effects of excess quantities of herbicides occurred on the Rogue River, Oregon in 1977 (Lorz et al, 1979). In this case, a large quantity of treated irrigation water containing Magnicide H, a gaseous form of acrolein, was released into the river within 24 hours of treatment instead of the recommended holding time of 6 days. The Oregon Department of Fish and Wildlife estimates that a ten mile section of river was affected, that 238,000 fish were killed, including 42,000 salmonids with an estimated value of $284,000. Acrolein is used to control aquatic vegetation growth, and is highly toxic in its normal applied concentration.

The Washington State Department of Ecology published the results of a pilot project conducted in the Snohomish River Basin (Ward, 1999). The purpose of the project was to test a short list of regional salmon habitat indicators using existing data from a pilot watershed located in Washington State. The Snohomish basin was selected because large amounts of data had previously been collected compared to other existing watersheds. A work group consisting of select state agencies identified 15 indicators in 5 functional categories: Fish Abundance, Water Quality, Water Quantity, Land Use/Cover and Physical Habitat. The study group broke Water Quality down in to three categories, only one of which of contained data considered “usable” for inclusion in this report: temperature. Biological Water Quality was the second subcategory, Chemical Water Quality was the third subcategory, and it looked at the “Percent of water rated excellent, good, fair, poor - possible parameters would include temperature, dissolved oxygen, biological assessment demand, pH, ammonia + nitrate, nitrogen, total phosphorous, total suspended solids, and bacteria to produce a single number.” Several plots were developed using existing data from prior reports issued by the state, however, the authors concluded that due to the low number of assessed waters, this indicator was not converted into a rating of any kind. In effect, the Snohomish River Basin report completely overlooks any kind of meaningful analysis of biological or chemical toxins on the immediate spawning habitat of Pacific Northwest Salmon. This gigantic northwest watershed presents itself as an ideal candidate for further studies in applied mycorrhizal mediation techniques, since the natural terrain is ideal for mushroom habitat, and because a huge number of returning adult salmon seek these headwaters for spawning. In my literature research for this thesis, it was common to run across these types of government reports, that seem to stop just short of making the next step toward taking or recommending really common sense activities to help these natural habitats get back to pre-human intervention conditions.

The Puget Sound Conservation and Recovery Plan (Office of the Governor, 2005-2007), was developed by the state legislature and approved by the governor of the State of Washington to identify and focus on the goals, strategies, funding and specific measurable results for protecting and conserving Puget Sound. The following priority areas were identified along with the specific sub-tasks required to support them and the identification of the responsible agencies.

- Improve Water Quality in Hood Canal
- Clean Up Contaminated Sites & Sediments
- Conserve and Recover Orca, Salmon, Forage and Ground Fish
- Prevent Nutrient and Pathogen Pollution Caused by Human and Animal Wastes
- Protect Shorelines and Other Critical Areas That Provide Important Ecological Functions
- Restore Degraded Nearshore and Freshwater Habitats
- Reduce the Harm From Storm Water Runoff
- Reduce Toxic Contamination and Prevent Future Contamination

The governor's plan has the impact of providing opportunities for a broad spectrum approach to managing natural sites, ecological niches, and environmental management and sustainability. In spite of a nearly endless flurry of scientific studies, discussions, and arguments around how to define or characterize the natural world we live in, it seems fundamentally obvious that each day more and more opportunities are presenting them selves for using ecologically friendly, low cost and low impact methods for helping keep natural worlds intact and flourishing at every level of biological processing. By developing more effective methods and applications for introducing target fungal species to conditions that favor their growth, the possibilities for mycoremediation to become more prevalent in a variety of mediation and restoration roles seems reasonable as an ecologically beneficial symbiot. If we can plant a tree, why not plant a spore as well? As many native fungi have niches all along the watersheds and food webs of this state and others around the world, it is easy to envision a comprehensive study that defines the current state and presence of all known species and their symbiots, and through careful cultivation of those species arrive at some "optimal" or at least beneficial distribution goal that meets and supports the condition of so called sustainable agriculture and environmental management.

Restoration Ecology

Hammel, 1995, conducted laboratory experiments in an effort to isolate and define the specific metabolic pathways employed by lignolytic fungi that cause white-rot of wood and also degrade a wide variety of organopollutants. The ability of these eukaryotes to cleave fused-ring aromatics was generally thought to be impossible, or at least until now, unverified. "Recent lab results have shown that extracellular peroxidases of these fungi are responsible for the initial oxidation of PAH's. Fungal lignin peroxidases oxidize certain PAH's directly, and fungal manganese peroxidases co-oxidize them indirectly during enzyme-mediated lipid peroxidation (Hammel, 1995)." The results of this study were suggestive of the role played by white-rot fungi in these reactive processes but work continues on a fully developed, conclusive mapping of these metabolic processes. The researcher does concede that lignolytic fungi make attractive candidates for use in low technology bioremediation programs. Although these organisms are slow reactors, they occur naturally in soil litter and are highly nonspecific in their action. We still know little about the specific mechanisms used by white-rots for organopollutants oxidation.

In 1992, article 2 of the Convention on Biological Diversity (Zerbe & Kreyer, March 2006) defined "biodiversity" as "the variability among living organisms from all sources including, *inter alia,* terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems." Biodiversity has recently become a common topic in the field of environmental studies and applied sciences, specifically as it relates to land use, nature conservation, landscape development and habitat or ecosystem restoration. Human presence and influence in the environment has had effects that contribute both to the increase of biodiversity and the decrease of biodiversity. Humans regularly introduce non-native species into some areas, while they destroy species through the destruction or degradation of their natural habitats in others.

"Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed. The goal of restoration is to create a self supporting ecosystem that is resilient to perturbation without further assistance" (Ruiz-Jean & Aide, 2005). The criteria for restoration success should be clearly established to allow meaningful evaluations of restoration projects. The Society of Ecological Restoration (2004) produced a primer that provides a list of nine factors that should be used to clearly provide a measure of restoration success. After evaluating 468 articles that met the study criteria for site evaluation or restorative success, only 68 were found to have conducted follow-up studies to evaluate restorative success (Ruiz-Jean & Aide, 2005). Of these 68 studies, none were found to address the full cross section of categories deemed necessary by the SER criteria established in their 2004 report. They conclude that ideally, all projects would follow the guidelines set forth in the primer. Cost and budget were the most common limiting factors. The 9 factors presented were: (1) similar diversity and community structure in comparison with reference sites; (2) presence of indigenous species; (3) presence of functional groups necessary for long-term stability; (4) capacity of the physical environment to sustain reproducing populations; (5) normal functioning; (6) integration with the landscape; (7) elimination of potential threats; (8) resilience to natural disturbances; and (9) self-sustainability. Although measuring these attributes could provide an excellent assessment of restoration success, few studies have the financial resources to monitor all these attributes. Furthermore, estimates of many attributes often require detailed long-term studies, but the monitoring phase of most restoration projects rarely lasts for more than 5 years.

Rivers, streams and riparian zones have been subject to the degrading and toxic effects of human activities and presence worldwide since the dawn of civilization. Factors like floodplain inundation, groundwater recharge, and organic matter exchange adversely affect structural and functional diversity in streams, and reduce water quality for humans and other biological organisms that depend on these environments for their success. Where restoration attempts have been made, they are generally poorly documented, and often ignored, particularly when projects fail, or reflect poorly upon local, state and federal government. Moerke & Lamberti (2004), surveyed government, private and non-profit organizations in the state of Indiana in an attempt to evaluate successes of their designs. Only 10 projects were evaluated and involved relocations, daylighting, and floodplain reconnection projects. Most involved aesthetic type goals, and were vaguely defined. The importance of in-stream aquatic biota was considered in only 50% of the projects.

Vorass and Portele (2001), submitted their report to the State of Washington on contaminated sites and the Endangered Species Act (ESA), pointing out that the state's regulatory criteria for prioritizing contaminated sites cleanups are based largely on health risks to humans. Their report to the Department of Transportation (DOT) was designed to provide an internal assessment tool for the DOT to assess and prioritize contaminated site risks to the Endangered Species Act listed aquatic species. The scientists came up with a three tiered evaluation process to determine relative potential for contaminated sites to affect ESA – listed fish species. Tier I evaluated sites for their relative distance to the documented presence of listed fish and their critical habitat. Tier II evaluated the status of hazardous material releases and the potential for contaminants to impact surface water and critical habitat areas. Tier III assigned a quantitative site scoring to rank sites based on risk to ESA – listed fish species. Scores range between 0 – 100 with higher values assigned to higher risk sites. Out of 103 evaluated sites, 41 were considered to pose potential risk and received quantitative scores. DOT will seek funding for cleanup of the 16 sites receiving scores of 75 or more. The remaining sites will receive further assessment to determine if additional cleanup efforts are warranted. The WSDOT plans to use this model for future site evaluation and characterization activities, and whether or not individual sites should become candidates for restoration.

Vorass and Portele (2001) conclude that although there remains some uncertainty in the area of relative toxicity of individual contaminant levels on fish species, the model is considered useful as an overall tool for the regulated community and agencies in establishing supportable cleanup decisions for any controlled site in

Washington. Restoration ecology and sciences, including bioremediation and mycoremediation, provide ideally suited, bio–friendly applications for the kind of sites that DOT typically encounters. Along the Pacific Coast of North America, literally from California to Alaska, native fungal species thrive in moist, low light riparian zones that double as habitat for a variety of marine species, including migratory salmon, shellfish, etc. The entire web of life becomes candidate for the naturally enzymatic processes that many plant species employ, particularly as alternatives to dredging, and other more disruptive methods. Even at the beginning of new, emerging research on fungal life forms, the use of native life forms presents a comforting sort of presence and consequence that future research and practice may eventually illuminate ways for the fungi to be effectively used in bioremediation, and practical and commercially viable ways.

D'Annibale et al, 2006, investigated the role of autochtonous filamentous fungi in the bioremediation of sites historically contaminated with aromatic hydrocarbons. The study looked at nine strains of fungi isolated from a heavily contaminated and aging soil to assess their potential for degradation. Among these strains were Allescheriella sp. Strain DABAC 1, Stachybotrys sp. Strain DABAC 3, and Phlebia sp. Strain DABAC 9 – all were selected for remediation trials and tested for their abilities to grow under nonsterile conditions and to degrade various aromatic hydrocarbons in the same contaminated soil. After 30 days, fungal colonization was obvious to the naked eye and confirmed by ergosterol determination. Although pH conditions were below normal alkalinity levels, and heavy metals were present in much higher than normal quantities, the fungi still produced laccase and Mn and lignin peroxidases. All of the selected isolates resulted in marked removal of naphthalene, dichloroaniline isomers, 0-hydrosybiphenyl, and 1,1'-binaphthalene. Overall, these autochthonous fungi led to a significant decrease in soil toxicity, as assessed by both the Lepidiam sativum L. germination test and the Collembola mortality test. The study also suggests that biological treatment technologies for the remediation of soils, groundwater, and riparian zones are becoming widely known for their ability treat toxic sites and remain environmentally friendly relative to secondary impacts due to their presence, whether they are introduced by soils managers, or occur naturally as part of the plants own spore dispersion mechanisms (D'Annibale et al, 2006): "Sites contaminated by recalcitrant organic compounds have often been shown to be characterized by the concomitant presence or heavy metals. In such a difficult case, the use of a filamentous fungus (white rot fungi, in particular) may give some advantages over bacterial bioaugmentation. Fungi display a high quality to immobilize toxic metals by either insoluble metal oxalate formation, biosorption, or chelation onto melanin like polymers. Moreover, due to the low substrate specificity of their degradative enzyme machinery (e.g. laccase, lignin peroxidases, and Mn peroxidases), fungi are able to perform the breakdown of a wide range of organopollutants in contaminated soils." (D'Annibale et al, 2006). Researchers are discovering that lignolytic peroxidases production is no longer the unique prerogative of white rot basidiomycetes (Field et al, 1993). More and more studies are reporting the presence of these enzymes in other fungal taxonomic groups, the authors report (Ayed et al, 2004).

Many prior studies in mycoremediation have been performed on artificially contaminated soils in more or less sterile conditions, suggesting the importance of investigating the use of fungal remediation in soil from real sites, helping to make them more transferrable to field scale studies and applications (Pointing, 2001). In D' Annibale et al, 2006, the authors study the aged and contaminated soil from a large, decommissioned chemical industrial site called ACNA, in Savonna Italy. Large scale production of a variety of industrial chemicals had gone on here for over 100 years, including the production of many organic chemicals. It was officially shut down in 1994 and is characterized by the presence of aromatic hydrocarbons, including chlorinated benzenes and anilines, thiophenes and polyaromatic hydrocarbons and heavy metals. The authors of this research paper engaged in a joint study project known as the Sisifo Project, which used various methods to test remediation strategies and methods that might prove effective at this specific location. One result of this effort that led D'Annibale and her colleagues on their quest, was the failure of several indigenous bacterial prokaryotes who were reported to be highly specialized in the catabolism of several aromatic compounds. This program failure led the researchers to pursue special interest in the assessment of indigenous fungi from the site, followed by their reinoculation as a technically feasible and promising approach to bioaugmentation. In their own words "The aim of the present work was to screen for the aromatic hydrocarbon degrading potential of fungal strains isolated from the ACNA soil and to assess the possible use of such selected autochthonous fungi in an ex situ soil biotreatment via bioaugmentation.“ In this study, all of the selected isolates were able to colonize the amended ACNA soil under nonsterile conditions which suggests they have tolerance to high concentrations of toxic contaminants while at the same time demonstrating their ability to compete with the indigenous bacterial microflora. The final conclusion of the authors is that "…this study confirms that the isolation of fungi from a contaminated soil followed by their reinoculation at the same site can be a valuable remediation strategy (D'Annibale et al, 2006)." Scientists and other environmental planners do, however, need to pay attention to many influencing factors in their selection, or mix, of bioremediation strategies.

About the Fungi and Factors Relevant to Their Selection and Application in Bioremediation Strategies

In order for mycorrhizal bioremediation techniques to be applied successfully in the treatment of xenobiotic contaminated soils, a number of factors must be considered early in the planning stages. These include the identification and implementation of physiochemical and nutritional conditions that favor the growth and xenobiotic degradative behaviors of indigenous or inoculated xenobiotic degrading microbes. One commonly applied bioremediation technique is biostimulation, which involves the selection of microbes based primarily on their xenobiotic-degrading abilities, using indigenous organisms that are already resident at the site. Another technique is bioaugmentation, which involves the use of organisms with superior pollutant degrading qualities and involves inoculating a contaminated site with organisms from other sites or spawn locations. By selecting attributes such as higher growth rates, competitive ecological strategies, tolerances to higher contaminant concentrations, specific nutritional capabilities, and pH or temperature growth optima, it may become possible to obtain dominant colonization and remediation outcomes or successes (Lamar et al, 1999).

Extensive laboratory study of a group of wood decay basidiomycetes, collectively called "white rot fungi" has shown their unique ability to degrade or assist in the degradation of a wide variety of contaminants and toxic compounds. This ability makes them ideal candidates for their applied use in contaminated soils, particularly those contaminated with complex mixtures of hazardous chemicals. A number of field studies using the bioaugmentation approach have demonstrated the effectiveness of using fungal treatment of pentachlorophenol (PCP) contaminated soils (Lamar, 1999). Soils contaminated with both PCP and creosote, which tends to have high concentrations of polyaromatic hydrocarbons (PAH's) have also been successfully treated. Most of the work on pollutant degradation and soil remediation by white rot fungi has focused on only a few species, predominantly *Phanerochaete chrysosporium* (Singh, 2006).

Lamar et al. (1999) concluded that identification of the most effective fungus for a particular set of contaminant-soil conditions are critical to the successful application of fungal bioaugmentation. Fungi would be selected based on their biochemical, physiological, and ecological attributes that offer them superior performance potential under a given set of contaminated media or soil/substrate conditions.

In their 1996 report, Lestan and Lamar investigated the development of fungal inocula for bioaugmentation of contaminated soils by inoculating soils contaminated with hazardous organic compounds. Their study used pelleted solid substrates coated with a sodium alginate suspension of fungal spores or mycelial fragments that were incubated until overgrown with the mycelium of selected lignin-degrading fungi. Evaluations were conducted using *Phanerochaete chrysosporium, P. sordida, Irpex lacteus, Bjerkandera adusta, and Trametes versicolor.* These inocula were selected because they resisted competition and proliferation from indigenous soil microbes, were lower in moisture content than current fungal inocula, and possessed enough mechanical strength to allow handling without a significant change in the mechanical consistency of the pellets. Following inoculation at a rate of 3% in artificially contaminated nonsterile soils, *I. lacteus, B. adusta, and T. versicolor* removed 86, 82, and 90% respectively of the pentachlorophenol in 4 weeks.

Lestan and Lamar, 1996, claim "…the salient feature which makes lignin-degrading basidiomycetes attractive as potential microbial agents is their ability to degrade a wide variety of hazardous compounds." Many of the obstacles or impediments that face many users of organic compounds and plant materials face scientists and technicians involved in the development, production, inoculum formation, transport, delivery, and application to the soil or site substrate. Getting the goods to market in a viable condition presents a host of challenges. The authors cite the presence of extensive literature on the effectiveness and suitability of different types of carriers of fungal inocula for biological control, fungal spawn, introduction of mycorrhizal fungi, and bioaugmentation. Most current methods for delivery involve the use of wheat straw, corn cobs, wood chips and commercial mushroom spawn that are completely grown through with the mycelium of the selected fungi. Selection of the proper substrate is important to ensure sufficient nutrient reserves to support the colonization of the target contaminated soil or distribution site. Problems that face commercial and scientific applications of fungi include low inoculum potential, requiring large quantities by weight of the initial spawn material, and "flashing." Despite modern refrigeration techniques, this phenomenon occurs when metabolic temperatures reach high enough levels, that they may kill the living organism or radically slow the growth process. Destruction of the fungal biomass also occurs as a result of this type of temperature rise. Lestan and Lamar 1996, focused on the effectiveness of inocula in removing pentachlorophenol [PCP], and suggest that the development of low cost methods of engineering and sustaining the health of the fungal inocula during transport is key to the success of this emerging science as a practical method of bioaugmentation of soils and other target sites, like riparian zones.

Lestan and Lamar conclude their 1996 study with the following recommendations for overcoming some of the logistical and distribution problems that manufacturers and others interested in scientific application of mycoremediation techniques. One popular method that is currently used by mushroom growers is simply to “bulk up” pure cultures using lots and lots of substrate material or agricultural waste, like corn cobs. These methods tend to be labor intensive, and the quality of the inoculum produced is often variable and of questionable quality or consistency. One way of overcoming this problem, and others, is the use of pelleted fungal inocula: a pellet core, usually made of wooden dowels or plugs, each of which contains a carrier, nutrient source, binder and some type of lubricant. This plug is then encapsulated by a layer of mature fungal mycelia. This technique produces a highly controllable product, as each pellet has a specific ratio between the amount of fungus and the substrate for growth and activity.

This allows for easily predicting the amount of growth and pollutant degrading activity from each inoculum unit [pellet] and provides a direct measure for quality control and “batch” evaluations. Another measure that factors in the importance of the transport and distribution of fungal inocula is mechanical strength, which aids in the collection, packing and final application processes. This is especially critical in soil applications where the rubbing frictions and grinding action of the soil may break down and reduce the inoculum potential. When the external coating of mycelium is broken down mechanically, it allows for entry by competing organisms into the core nutrient and carrier elements of the individual pellets. This introduces a competitive advantage to local fungi and bacteria that may significantly reduce or overcome the intended beneficial effects of the introduced species.

Lestan and Lamar, 1996, conclude that lower moisture content will signigicantly reduce the costs associated with applied mycoremediation strategies by several avenues. This not only reduces overall mass and weight of the shipped product, but can also significantly reduce the volume, and provide enhanced storage characteristics of the inocula or spawn carrier. Pelleted inoculum was reduced approximately 50% in volume over the original mixture. On the side of weight or mass, the report goes on to suggest that a current inoculum formulation using nutrient-fortified grain-sawdust mix applied at a rate of 10% to a site with 100,000 cubic meters of contaminated soil would require 25,000 metric tons wet weight of inoculum, at 60% moisture. In this study, the average moisture content of pelleted inocula varied from 20.7% for an inoculum with *I. lacteus* to 35.5% for an inoculum with *T. versicolor*, about half that present in the fortified grain-sawdust mixture.

D'Annibale, et al (2006), observed that sites contaminated with persistent organic compounds are often characterized by the presence of heavy metals. In these cases, filamentous fungi may have specific advantages over bacterial bioaugmentation. Fungi display a high ability to immobilize toxic metals by either insoluble metal oxalate formation, biosorption, or chelation onto melanin-like polymers (D'Annibale, et al 2006). They are also able to break down a wide range of organopollutants in contaminated soils due to the low substrate specificity of their degradative enzyme machinery. The investigators in this study (D'Annibale et al., 2006) attempt to demonstrate the use of fungal remediation under non-sterile conditions and with soils from real contaminated sites, making the results potentially transferable on a field scale.

D'Annibale et al. (2006) reviewed and assessed the feasibility of ex situ bioaugmentation with allochthonous fungi on aged, contaminated soil. The site is a former industrial area where a wide array of organic chemicals had been deposited for over 100 years. They included aromatic hydrocarbons, chlorinated benzenes and anilines, thiophenes, polyaromatic hydrocarbons and heavy metals. Previous coordinated project attempts to show the success of using indigenous prokaryotic bacteria specialized in catabolism proved unsuccessful (Duran and Esposito, 2000). The failures prompted the authors of this study to assess the eventual presence of yeasts and fungal microbiota adapted to the historical contaminants of this site for use in its remediation. The aim of this work was to screen for the aromatic hydrocarbon degrading potential of fungal strains isolated from the site and to assess the possible use of such selected autochthonous fungi in an ex situ soil biotreatment via bioaugmentation. After an extensive series of biochemical tests and statistical analyses, the authors confirmed that the isolation of fungi from a contaminated soil followed by their reinoculation at the same site can be a valuable remediation strategy.

The use of lignin degrading basidiomycetes for remediation of soils contaminated with hazardous organic compounds has been studied a great deal (Davis et al, 1993). What makes these organisms attractive is their ability to degrade a wide variety of compounds (Lamar et al, 1992). In spite of this knowledge, the development and use of fungal bioaugmentation or remediation on an industrial or wide-spread scale has been inhibited by inconsistent treatments in the field. There are many known carriers of fungal inocula that have been demonstrated to be effective in laboratory and field studies: peat, granular vermiculite, grains, alginate pellets, wood chips, straw, corn cobs, etc. Problems that have been identified with large scale production, maintenance and delivery include, cost, inconsistent quality, drying, flashing (production of excess metabolic heat), and destruction during the application process.

Zerbe and Kreyer (2006) in their prelude introduction to the fourteenth issue of The Journal of Society for Ecological Restoration International, point out that the term “biodiversity” should read more or less as follows, by way of arriving at some standard and clear definition of the concept: “The variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part, this includes diversity within species, between species and of ecosystems." As an overall topic then, biodiversity is becoming more important in biological and ecological research aimed at practical applications like land use, conservation of natural environments, development and restoration.

About the Toxins

A significant portion of the detailed supporting activities identified in this literature review include things like cleanup of mercury, poloybrominated biphenyl ether, (PDBE or flame retardants), and other chemical toxins, oil spill management, storm water runoff, contaminated sites and sediments, sewage management, nutrient and pathogen pollution caused by human and animal wastes. Mycoremediation techniques both in and outside the laboratory have shown promise as applications for mediation of all these types of impact factors throughout the world.

In 1994 the Washington State Pesticide Monitoring Report (Davis & Johnson 1994) issued the results of their study of 24 pesticides and breakdown products detected in fish tissue, and nine compounds detected in sediment samples collected from seven freshwater sites in 1992. Pesticides detected in high concentrations included forms of DDE, DDT, dieldrin, and heptachlor epoxide. DDT and metabolites chlordane, and two PCB's were detected at all sample sites.

High concentrations of DDE were found in whole large scale suckers from the Yakima River and Kokanee fillets and eggs from Lake Chelan. Lipid normalized values indicate that the bioavailability of DDT and metabolites to fish in the Walla Walla River is higher than the other sites tested. EPA human health screening values, used only to prioritize problem areas, were exceeded for DDT in fillet samples from Lake Chelan, Crab Creek, and the Yakima River. Screening values for total PCB's were exceeded in samples from Lake Chelan, Crab Creek, Yakima River, and Mercer Slough. Dieldrin in fillet samples from the Yakima River and heptachlor epoxide from the Walla Walla River were also above screening values. Sites with high fillet samples exceeding the National Toxics Rule (NTR) criteria were recommended for addition to the 303(d) water quality limited list.

Comparisons with historical data indicate that pesticide concentration in fish tissue have changed little. DDE in samples from the Yakima and Walla Walla Walla Rivers continue to be above proposed wildlife criteria. Concentrations of DDE in fish from the Walla Walla and Yakima Rivers, and chlordane in the Yakima and Mercer Slough are ranked high as compared to national averages. Pesticide levels in sediment from the Yakima River appear to be decreasing as compared to samples collected since 1984.

Washington State issued its Sediment Cleanup Status Report in June, 2005. The report summarizes all known or suspected sediment cleanup sites in Washington State. Sediment is defined by the report as "particles of dirt that are discharged or washed into and travel through water systems to precipitate out onto sea floors and lake and river beds. Many contaminants adhere to these solid particles on their journey so that sediments, by nature, concentrate contamination in the areas where they collect. It is that muck that squishes up between your toes when you run out into a lake to go swimming…" The contamination found in many types of sediment results from the release of harmful contaminants into the aquatic environment from industrial sources, municipal discharges, commercial activities and accidents. This report estimates that between 2,940 and 3,340 sites contain contaminated marine sediment. Several challenges facing cleanup or remediation are cited by the report: Disposal Capacity and Cost, Uncontrolled Sources, Reluctant Liable Parties, Resources, State-Owned Aquatic Land Management Policy, Regulatory Uncertainties, Potential Superfund Listing, Area-wide Contamination. This document is extensive in its listing of contaminated sediments sites, but contains little data on what is being done and how to correct or mediate the presence of these substances in our environment. It is primarily a database or catalogue of sites and agency links that may or may not reflect accountability and responsibility for making measurable progress in achieving results in the actual cleaning or remediation of these areas.

Ruckleshaus and McClure (2007), in their ecosystem-scale study of Puget Sound, conclude the following with respect to toxic contaminants: "Contaminants – toxins, nutrients, pharmaceuticals and pathogens – entering Puget Sound accumulate in sediments, marine waters and organisms and negatively impact biological populations, ecosystem integrity, harvest availability, and human health." Toxins introduced in Puget Sound are appearing in high level concentration in upper level predators like salmon, seals and orcas in spite of having been banned decades ago. The number of highly contaminated acres has come down due to clean up efforts, but some contaminants, like PCB's are declining slowly, if at all, and PAB's, polycyclic aromatic hydrocarbons have increased in long term sediment monitoring stations in Puget Sound. The authors conclude that reduction of inputs of toxic compounds to the area will benefit ecosystem and human health in general. Remediation and restoration activities designed to clean up legacy locations should be applied to help reduce the amount of toxins that move back into the food web.

An exploratory report issued by the Washington State Department of Ecology (Seiders et al, 2003) in support off the Washington State Toxics Monitoring Program, investigated the occurrence and concentration of toxic contaminants in edible fish tissue and surface waters from freshwater environments in the state. The program was started in 2001 as a result of increased public and scientific concerns over the dramatic increase in contaminants in the environment of Puget Sound. Twenty five samples of fish from eight species were collected from ten separate sites. Contaminants found included mercury, PCB's, dioxins and furans, flame retardants (PBDE's) and chlorinated pesticides like DDT and its metabolites, chlordane compounds, dieldrin, aldrin, Beta-BHC, chlorpyrifos, endosulfan sulfate, heptachlor epoxide, hexachlorobenzene, lindane, and mirex. Tissue samples from eight of the ten sites exceeded National Toxics Rule (NTR) for the protection of human health. Water samples from ten sites were found to include six pesticides at low levels and frequencies, including bromacil, dichlobenil, atrazine, diuron, hexazinone, and terbacil. The report recommended continued evaluation and monitoring of potential health risks to human health from consumptions of contaminated fish and the addition of eight sites to Washington's 303(d) list. The report suggests that, for many areas of Washington State, information is not available that shows levels of toxic contamination in freshwater fish and surface water, as they are both present in riparian zones throughout the state. These chemicals can be extremely persistent, as they not easily broken down at the biochemical and molecular levels, resulting in the presence and accumulation in the environment for decades. This environmental presence results in biomagnifications and bioaccumulation in organisms that engage in the food chains, or web of life, thereby expanding and distributing the impact of their presence.

The harmful effects are carried away from the point source of the pollutant and find their way into the biochemistry and metabolic pathways of both higher and lower order life forms. Some of the problems that result on various wildlife and humans involve behavior, neurological, and reproductive abnormalities. The report cites Washington State Department of Health statistics that list 16 site specific consumption advisories for finfish and shellfish in Washington due to contamination by mercury, PCB's, chlorinated pesticides, and other metals and inorganic chemicals. The state toxics monitoring program is seen as one method of relieving an absence of meaningful monitoring and science that is directed at targeting and managing existing toxins in our environment. Naturally derived techniques like bioremediation and mycoremediation offer themselves as viable alternatives, at low cost and low impact, for planning agencies to use when devising or recommending methods for reducing or eliminating the presence of these harmful toxins.

Past monitoring efforts in Washington have detected toxic contaminants in surface water, sediment, and aquatic animal tissues. Efforts to monitor and manage toxic chemicals in freshwater fish tissue, sediments and water have unfortunately declined over the last decade due to budget cuts. In 2000, renewed concerns resulted in the establishment of the Washington State Toxics Monitoring Program. Among their stated goals are: exploratory monitoring of new instances and locations of contaminants in freshwater environments; establishment of communications methods to disseminate date to citizens and resource managers; trend monitoring; and cooperation with other like groups and agencies.

Fungal Metabolism of Polycyclic Aromatic Hydrocarbons

Mycoremediation lends itself readily to the cleanup of many toxins, in particular, a group known as PAH's or polycyclic aromatic hydrocarbons. These pollutants have been detected and observed in many terrestrial and aquatic ecosystems, including riparian zones. PAH's find their way into our environment from a number of sources: incomplete combustion of fossil fuels, shale oil and cigarette smoke, discharge of petroleum and coal gasification and liquefaction, incineration of wastes and agricultural and forest residues. The molecular constituents adhere to suspended particles and are transported into soils, siltation and sediments of river systems and estuarine zones (Singh 2006). Because they are hydrophobic in nature, and possess low vapor pressures, PAH's have a strong affinity for absorption and accumulation in sediments. They are also very stable thermodynamically, due to strong negative resonance energy. The half-lives of the heavier rings are on the order of years in most ecosystems. All these factors contribute to the persistence and threat posed by these compounds to all living organisms. PAH's exhibit toxic, mutagenic, tumorigenic, and carcinogenic properties. Recent studies have shown that metabolic activation of PAH's to electrophilic species form covalent bonding with nucleophilic groups of DNA, and result in mutations. This poses a significant risk to humans (Singh 2006).

In "The Fungus Among Us" the authors, Aust and Benson, 1993, examine the potential for practical applications of scientific knowledge that is emerging throughout the field of toxicology and microbiology from an economic perspective, with some brief background on the biochemistry behind this economic. The authors argue that with estimates for hazardous waste remediation and superfund site cleanup ranging from between $0.5 and $1 trillion, that the application of naturally occurring and native species with bio-degradative qualities is not only eco-friendly, but cost effective also on a relative scale. One advantage the authors point out for white rot organisms is that they tend to be "…nonselective in degrading all the chemical components of complex mixtures. For example, all the components of Aroclors (PCB), toxaphene, creosote and coal tars are degraded by fungi. In some cases this is related to the nonspecific nature of the peroxidases secreted by the fungi, and in other cases it is related to the variety of mechanisms that the fungi use to degrade chemicals." The authors conclude their argument by pointing out that fungi can be grown on very inexpensive agricultural and forest wastes like sawdust and corn cobs, garbage in the minds of most people, but with high potential as recyclable carbon materials. In this case, they would serve as the food source for sustaining, transporting, and inoculating cultures in select applications. And, relatively cost effective mass production techniques have already been developed for commercial spawn runs like common button mushrooms, and other culinary/medicinal varieties. One of the great advantages of using native species in applied ecological remediation solutions is they produce many of the secondary and tertiary elements needed for beneficial symbiots to thrive as part of their natural growth and development processes.

One of the challenges facing successful applications of mycoremediation techniques in the field is the identification and selection of those organisms that possess the physiochemical and nutritional needs that will provide optimum growth and xenobiotic-degrading activities of the target toxin or contaminated soil site. In addition to selecting organisms with superior pollutant degrading qualities, the practitioner should also allow for selection of microbes bases on additional physical, biochemical and ecological characteristics that will lend themselves to high levels of performance. Simply stated, the preferred approach is to match the right fungal species with the right toxin, to achieve the desired result (Lamar, et al 1999). Some things to consider are superior growth rates, competitive ecological strategies, tolerances to high contaminant concentrations, specific nutritional capabilities or preferences, pH and temperature optima that lead to dominant colonization and finally the remediation of the contaminated soil volume by the introduced or inoculated organism. Recent laboratory studies of certain wood decay basidiomycetes, commonly referred to as "white-rot" fungi, have clearly shown that they possess the ability to degrade a wide variety of contaminants. Most of the work on pollutant degradation and soil remediation has focused on only a few fungal species (Lamar, et al 1999).

As more and more research is done on the effectiveness of using mycoremediation as a practical application for removing toxins from contaminated soil sites and other ecological systems, including riparian zones and urban or industrial sites contaminated with process wastes, scientists are recognizing the importance of choosing the most effective fungus for a particular set of conditions. In order to facilitate the optimum growth and xenobiotic degrading activities of the bioaugmentation, fungi should be selected on the basis of the "best match" for the site, according to some contaminant remediation profile. Again, pick the right fungus for the job that lies before you. These treatability studies can be quite extensive and laborious. For example, tolerance to contaminant and hyphal extension rate did not prove useful when attempting to predict the contaminant degrading capability of a fungus for degrading PAH's in wood. The most effective fungi for degrading PAH's in wood turn out to be the brown-rot basidiomycetes, *G. trabeum*, and the dueteromycete *S. circinatum*, especially for the lower molecular weight toxins.   These organisms both exhibit low hyphal extension rates and are sensitive to creosote. So, it appears that basic measures of contaminant degrading capability or tolerance to a pollutant can not alone provide enough information for selecting fungi for use in bioaugmentation of contaminated soils.

More information on the bioremediation performance of well characterized fungi on soils that represent a range of soil physical, chemical, and biological characteristics and pollutants is needed to build a database of information that can be used to select superior fungal strains to evaluate for application to specific contaminant-media conditions (Lamar, et al 1999). Forthcoming studies concerning fungal contaminant degradation in complex media should include in their design the additional purpose of adding information to such a database.

Alternatives to Mycoremediation

How does the state's primary cleanup agency currently define the known world of sediment cleanup sites? In their 2005 report, DOE identified 142 specific areas with enough reliable data to consider them cleanup sites. Of these sites, 115 are in or near Puget Sound, and 27 are located in freshwater environments. There is a renewed emphasis on assessing these freshwater "riparian" environments as more is learned and understood about the accumulation and distribution of toxins throughout the biosphere. These sites should be considered ideal candidates for mycoremediation techniques and applications, based on their mutually supportive habitats for both terrestrial and aquatic fungi, as well as fish species and other wild organisms that support the carbon chain.

What are some of the methods currently employed by the State of Washington to cleanup, restore, or mediate sites that have been identified as or meet the established criteria for cleanup? In their 2005 report, the Washington State Department of Ecology proposed to answer this question. From a strictly regulatory perspective based on agency responsibility, the following picture emerges. Sediment cleanup is primarily under the authority of either federal Comprehensive Environmental Response Compensation Liability Act [CERCLA] as controlled by the U.S. Environmental Protection Agency [EPA] "Superfund" program or via state cleanup laws and rules. The state authorities are the Model

Toxics Control Act cleanup regulation, Chapter 173-340 WAC, and the Sediment Management Standards, Chapter 173-204 WAC.

Challenges

Many of the challenges that are faced by planners and policy makers when addressing cleanup, restoration, and mediation of marine, freshwater, and terrestrial environments may be largely overcome someday, or at least to some beneficial level, by employing mycoremediation techniques. Some of these obstacles and outcomes are presented below, along with their advantages and disadvantages. One method that is often used to mitigate the effects of toxins and other contaminants that appear as the result of human activities is simply to remove the contaminated material from the site. Once removed, however, an alternate disposal site must either be selected, or some other method of reducing the compounds to a non toxic state must be employed. One solution has been to burn the subject material, which causes a host of secondary problems of its own.

Often the toxins are not destroyed, but simply redistributed as terrestrial and atmospheric contaminants which may become distributed on a global scale due to naturally occurring atmospheric and other distribution effects. One of the obvious advantages of mycoremediation is that the naturally occurring enzymatic processes employed by certain fungi actually alter or reduce the offending toxins to benign biochemical compounds and elements that can simply be left in place, overcoming the need to dredge, remove or otherwise dispose of and manage the original offending compound. This was one of the benefits noted when the common Oyster Mushroom was used to reduce and breakdown PAH's that were introduced into the marine waters of the Pacific Ocean following the Exxon – Valdez oil spill. The hydrocarbons were actually broken down to into harmless carbon constituents at rates approaching 75 – 85 percent (Bonaventura & Johnson, 1997).

The importance of selecting an appropriate application method for the problem at hand is reported on by Sasek, et al (2006) in their comparison of two biodegradation methods using composting under controlled conditions and treatment with ligninolytic fungi. The target chemical was synthetic polymers sourced from used beverage bottles. Two types of copolymers were tested: polyester-amide and aromatic-aliphatic. Synthetic polymers have the characteristic of being resistant to microbial attack, and tend to persist as contaminants in the environment wherever and however they are introduced. The experiment was run using standard raw materials under controlled conditions to help eliminate the introduction of confounding factors that are common in field studies, where control of contaminants and other xenobiotic organisms is difficult. Ligninolytic fungi were selected for three reasons: they are the only organisms capable of efficient degradation of lignin, the most resistant biopolymer in the environment; they have shown the ability to decompose many human derived organopollutants and; the ability of several fungal strains to attack some resistant synthetic polymers is documented (Sasek et al, 2006). Composting proved to be the most effective "reducer" in all measures but one in its effect on reducing molar mass of the original sample. One explanation offered for this result is the short duration of the inoculation period for the fungi, at 32 days and room temperature.

Bioaccumulation has been observed as a bioremediation process where living mycelium and fruiting mushroom bodies take up heavy metals into their flesh to a considerably higher level than other agricultural crop plants (Oghenekaro et al, 2008). Heavy metals are natural components of the earth and occur, may in fact be necessary, at trace levels in many higher order organisms, including humans. They accumulate through the food chain, and may concentrate at high levels that pose a risk to the proper functioning of cellular and metabolic processes. The results of their study showed that when heavy metals are present, they influence the growth of white rot fungi and the subsequent release of enzymes that biodegrade xenobiotics. In their discussion, the authors conclude that *P. tuberregium* in particular, has the ability to accumulate heavy metals, making it a primary candidate for mycoremediation in polluted environments. Of particular interest might be follow on processes that can be applied to harvest and deal with the now highly toxified fruiting bodies.

In their report to the Washington State Department of Transportation, Thomas et al (1998) provided a comprehensive analysis of a mycoremediation project conducted in Bellingham Washington. The intent was to examine and contrast the effectiveness of three different biological approaches: mycoremediation, bioremediation, and enhanced bacterial remediation. The test site consisted of three excavated, aged, oil-contaminated soils stored at an open WSDOT maintenance yard in Bellingham Washington. Surprisingly, the results were not conclusive in distinguishing among the various treatments, as none met the prescribed criteria for success: attainment of the Method A Cleanup Level of total petroleum hydrocarbons (TPH) prescribed by the Washington State Department of Ecology (WSDOE) in Washington Administrative Code (WAC) 173-340 (WAC 1996) and Ecology Publication No. ECY-97-600 (WSDOE 1997) within the time period prescribed. The authors suggest, however, that the study program was valuable in understanding the variables involved in moving from a controlled mesocosm environment to large scale applications of bioremediation technologies and processes. The inconclusive nature of the results is likely the result of the vast heterogeneity of the test soils and the weathered condition of the petroleum hydrocarbons in the soil.

Uncontrolled Sources or Recontamination from Ongoing Efflux of Contaminants

This potential and very real problem is faced regardless of the mediation technique that is employed, and should be managed more from a policy and regulatory perspective. The idea here is that it makes little sense to engage in costly and complex methods for mitigating contamination of a site when it is known that some pollution source will continue to introduce contaminants that

will require more action in the future. In these cases, it makes logical sense of course to control the efflux prior to placing money into the cleanup effort. Even in this type of situation, however, there can be specific advantages to employing naturally occurring fungal species to a site while the lengthy machineries of government and policy making grind slowly to some permanent solution.
The introduction of a toxin specific fungal species can provide benefit simply by being allowed to run its natural bio-enzymatic course following an initial spawn introduction. As the mycelia spread, and the plant flourishes, the “mediation” advantage could be simply keeping the accumulation at bay, or at some reduced level, as politics runs its course. This can involve several years, or even decades as we all know, during which time the mushroom in its vegetative state quietly grows beneath our feet and alters or breaks down the harmful compounds. In theory, at some future point, more final and comprehensive plans can be implemented. This could be thought of as a tactical response to a known threat that costs very little to get started.

Reluctant Liable Party

In cases where there is reluctance by the liable party in a clean up or mediation event, time can be a factor. Negotiations are often stalled by an entity that does not see any advantage to cooperating with the cleanup, and may be fighting the outlay of large sums of working capital that they would prefer to see go into some other account or distribution. This may provide another window of opportunity for employing mycoremediation science up front, at low cost, that even the most intolerant party may agree to, since it can be viewed as good faith, kicked off with minimal or relatively low start up costs, and capable of providing real benefit to the environment. This can be viewed as beneficial to the offending or liable party, as their may be a public image promoted to reflect favorably upon them. In other words, we are taking some action, while discussions continue.

Resources

Lack of resources, or more accurately, not enough resource to go around, is a problem faced by nearly every entity in government, business, and virtually any agency of any type or make up. This presents another opportunity for employing simpler, low cost, front end activities that may become part of a host of multifaceted actions designed to recover or mediate disturbed habitats and recover from the introduction of toxic chemicals. This site would be an excellent place to test the practical application of biological uptake: the simple inoculation of a fungus that has an affinity for picking up arsenic and other pollutants that were generously spread around Puget Sound for decades on end, may have highly beneficial results for this abused ecosystem. If this fill were effectively impregnated with a mushroom spore and food source, of a variety that targets these local toxins, the downstream rewards could be significant.

State Owned Aquatic Land Management Policy

As more and more agencies become players in the field of environmental management of aquatic lands, sediments sites, disposal areas and long term remedies, the opportunity for mycoremediation applications presents the potential for an effective, eco-friendly technology for mitigation and sustainability of resources. Increased interagency coordination has re-emphasized the scrutiny and detail required to provide long term planning solutions for state owned aquatic lands and ecosystems. According to this report, Washington State has recently introduced a formalized methodology they call "collaborative management" which, in theory, results in more efficient cross functional communication between departments and agencies within the state's broad administrative network. Again, this should provide more opportunities for introducing mycoremediation techniques into the comprehensive planning efforts of legislators, policy and other decision makers.

As sustainability acquires an increased importance and focus at all levels of government administration, more and more research projects aimed at how to apply or most effectively employ processes in mycoremediation and bioremediation techniques will naturally lend themselves to planning efforts. Scientists and land use planners are recognizing that less is better. The natural balance that ecosystems strike for themselves is the "way of the way" for keeping the environment clean and efficient in terms of keeping the world green, and developing so called sustainable processes for all of our manufacturing and agricultural endeavors.

Potential Superfund Listing

Department of Energy site managers often delay or postpone actions on a given site when they anticipate the possibility of the site eventually becoming added to some broader and better funded cleanup activity like the federal Superfund. This was exactly the case for the Lower Duwamish Waterway and the East Waterway of Harbor Island. A more positive approach might be to implement in stages, as many larger programs are, clearly recognizing and identifying the later phases of the activity will be tied to out-year funding. For example, a pilot study using native fungal species in a confined site could be carried out using volunteers and scientific resources like graduate students or interns from forestry, fishery and other agencies with potentially common interests and strategic goals.

Area-wide contamination

Sites that have been identified as contaminated and treated as isolated cases, may be found to be part of a broader contamination source that is being included under a broader management program. If planners and site managers sit and wait for some potential action that rolls their site up into a broader funded program, much like the Superfund situation discussed above, then valuable time may lost, and a potential health hazard is left more or less unattended. Mycoremediation offers its self as a frugal means for intervening on a known hazard situation, and possibly defusing the threat potential in the near term.

Cost Estimates for Site Cleanup

In his book review of Biodegradation and Bioremediation by Martin Alexander, (Gealt, 1995) the author provides a broad variety of links to how humans might manage the disposal of wastes and ecological contamination using naturally occurring biotic organisms, like fungi and bacteria. At press time at least, they claim, some 32,000 hazardous waste sites existed in the United States alone, as of 1989 specifically.

In their 2005 report on toxics cleanup, the Washington State DOE developed a rough methodology for estimating costs associated with contaminated sediment cleanup. Out of 90 known contaminated sites, cost information was limited to or screened to include only the cost of construction and post-cleanup remedial action monitoring. The report confesses that these figures should be treated as order–of–magnitude estimates, since some information was either not directly available, or the sites were in varying stages of cleanup or construction. Other variances are introduced by the uncertainty of site boundaries, as yet undetermined or imprecise mediation remedies of strategies, and progress toward completion. In cases where the project has been completed in its entirety, actual data on costs experienced are easier to obtain and more reliable, since they are after the fact book recordings of real world cost experiences.

Remedies range from natural or enhanced natural recovery with monitoring, capping, dredging, *in situ* bioremediation and active treatment. Disposal options varied from near shore placement and confined aquatic disposal at regulated landfills. The report concludes that the options chosen in the various management action plans can significantly alter or impact the final cost estimates, and actual cost experience overall. Another factor that should be considered in this type of estimating, are the extended or subsequent costs and impacts, regardless of whether the current project or program budget is the cited or responsible authority. For example, simply moving contaminated material from one location to another, does not present a truly final solution to the toxic presence.

The contaminated material still exists, it is just in another physical location, and this may or may not be an overall improvement or remedy to the situation. This is one argument that was presented when the ASARCO smelter in Tacoma, Washington was originally tagged as a prime contributor to the presence of heavy metals in the soils and marine/freshwater environments of Tacoma and several neighboring counties. Many planners and politicians argued that, since much of the contamination is due to heavy metals, just leave them and they will sink to the bottom of the soil or marine substrates. Left undisturbed, covered over, or whatever, they pose no threat to the environment. This is similar to the argument that non-friable asbestos should just be left undisturbed, rather than attempt to remove or dispose of safely and cleanly.

The Department of Energy report presents the following cost categories in a tabled format: Out of the 90 sites that have associated cost information available, 56 are estimated at $5 million or less. Total cost for the 90 sites is roughly between $400 million and $1.2 billion. The variances, lack of good data, and errors in estimating make it clear that far better effort and attention needs to be applied to the area of mitigating the generation of toxins and other pollutants associated with any project, whether large or small. On the other hand, this knowledge begins to illustrate opportunities for many potential market and business niches that deal with waste management as a revenue source, and for newly applied science and technology in the field of bioremediation and mycoremediation.

Conclusion

In field settings, the ecologist or environmental planner is faced with the challenge of a diverse presence of arbuscular-mycorrhizal (AM) fungi that occur naturally, or without the intentional behavior of terrestrial modifiers like humans and other organisms. Bever, et al, 2001, examine this diversity and some implications of its presence as an agent in site restoration activities, whether planned for or not. Their study was aimed at finding multiple species of AM fungi in the field. Their initial examination of field soil was collected in 1992, and 11 species were identified, recognizing some margin of error based on sampling bias. Using a variety of entrapment techniques to refine their samples and obtain better counts, they arrived at a final count of 37 different species, one-third of which had not been described before. Recognizing that the presence of one species may promote the growth, health and well being of one plant, another may have just the opposite effect. For example, the presence of Ac. Collosica was negatively associated with soil phosphorous concentration while the opposite was true for Gi.gigantea.

The authors suggest that the entire plant and fungal community combine as a "superorganism" with complex interactions that support, enhance and evolve as an interrelated biotic system, with a web or network of communication and transport dynamics. The authors conclude by emphasizing the importance of reevaluations away from past understandings of plant succession, that include such process as even "below ground" organisms as dynamic participants to provide us a complete or better understanding of the many consequences of mycorrhizal mediation or intervention techniques in ecology planning and the intentional alteration of agricultural and riparian species that impact plant and animal life at virtually every level of the carbon cycle. Fungal species affect the ecosystem in the way they break down, build up, bioaccumulate, absorb and sterilize or destroy other organisms in the environment.

Bever et al, 2001, propose a model for viewing certain root symbiots, arbuscular mycorrhizal (AM) fungi, as key promoters of the general health of plant communities across the terrestrial biosphere. Specifically, they are critical in facilitating the ability of most plants to uptake phosphorous. This newly rendered version of plant ecology suggests many creative ways of employing these curious biota in landscape gardening, forestry, agriculture, and remediation of contaminated sites around the world. The authors performed a research study on a one-hectare grassland that had been abandoned as an agricultural site some 60 years. Their goal was to examine the presence of AM species in the field. 37 species were eventually identified, half of which have not previously been described. This number was much higher than the researchers expected.

Speculation is made that the high number of species may contribute to overall trophic responses of the native or host plants, as each will have differing requirements in a symbiotic relationship. The authors also suggest the total number plays some role in successional dynamics, as new plants migrate in over time from conditions of disturbance like fire. The paper concludes with another call for more research on the complex interactions that occur within soils and between plant ecologies. One confounding factor may have been the use of "trapping" methods – this involves the capture and return of small pots to the lab for examination over time, reasoning that certain species may not appear until seasonal changes alter environmental factors like humidity, etc. This allows for the introduction of error through cross contamination, and other vectors.

Another aspect of the benefits provided by the presence of mycorrhizal fungi in soils and marine systems relate to the trophic benefits that so called host plants experience. Rai, 2001 examined the use of microbial inoculants as a replacement for chemical fertilizers and pesticides. Estimates place the symbiotic partnership of plants and fungi at 90% of all known species. Among the established and theorized benefits are processes that result in the uptake of nutrients, reduced stress, improved nutrition, improved aeration, and soil structure. The author identifies several aspects of the technology that could be improved and recommends screening bioassays as an effective way to identify the most effective strain. The report concludes in general that the "hairy-root" technology has great promise for cultivation of target species and that the enzymatic processes that these organisms deliver to ecological planners are beneficial in not only breaking down toxins, but also as highly beneficial support members in most plant communities. This becomes more true as more data is collected and recorded on species interactions at the subsoil level.

Lamar et al (1999), noted that for the application of bioremediation techniques such as mycoremediation to be useful for mediating the impacts of xenobiotic contaminated soils, scientists and project managers must be able to identify and select for the introduction of species that will thrive in the physiochemical and nutritional conditions that are present in the target site or contaminated zone. The ultimate goal of this identification process is to obtain dominant colonization and ultimately remediation of a contaminated soil volume by the inoculated organism. Their research identifies a need for further investigations that will advance the application of this laboratory knowledge into the field, and eventually contribute to the development of physical processes that enhance mycoremediation techniques on a commercial scale. As this knowledge broadens, and more reference data is available to planners, the more practical and cost effective these specialty techniques in bioremediation will become.

Lamar's 1999 paper examined 20 fungal strains comprising six species of white-rot fungi on specific performance parameters. This report identified the most effective fungus for a given set of contaminant and soil conditions that one might encounter in the field, giving optimal growth and degrading activities, all of which are essential to the successful application of fungal bioaugmentation. Their work sets the stage for development of a universal, comprehensive reference database that would allow engineers and planners to apply the best available fungus and promotes its ultimate success as a mitigation technique that meets all the favorable attributes of being eco-friendly, benign, and transparent to users and other members of the biotic community, at all scales of magnitude and diversity. This study also illustrates, as the authors reflect in their conclusion, that simple measures of contaminant degrading ability or tolerance to a pollutant/s do not provide the complete picture for selection of fungi in bioaugmentation of contaminated soils or riparian zones.

As demonstrated by the case of *P. sordida*, one fungus may achieve superior performance under one set of conditions, and remain mediocre under another set of contaminant – media conditions. This statement is key to their work as well as the underlying thesis of this author's study: "More information on the bioremediation performance of well characterized fungi on soils that represent a range of soil physical, chemical, and biological characteristics and pollutants is necessary to build a database of information from which to base selection of superior fungal strains to evaluate for application to specific contaminant – media conditions." A database of this kind would include at least fungal characteristics like growth rates, growth temperature ranges and optima, sensitivities to and abilities to degrade specific contaminants, bioremediation performance in well characterized media and relative competitive abilities. This final factor is critical, and has been largely ignored in the scientific literature. A fungus must be able to survive a given set of conditions as well as degrade target toxins and pollutants.

In their report on environmental contamination, Bonaventura and Johnson, 2008, the authors examine environmental contamination sites that are challenging our "…global society to find effective measures of remediation to reverse the negative conditions that severely threaten human and environmental health." They define bioremediation as the use of "…microbes (bacteria, fungi, yeast and algae, although higher plants are used in some applications. New bioremediation approaches are emerging based on advances in molecular biology and process engineering." The report goes on to suggest that these methods are favored from the perspective of environmental impact as the outcomes of these processes avoid the production or regeneration of microbial pathogenesis. As a relatively benign and inexpensive contributor to a entire spectrum of mediation approaches to contaminated ecosystems, the report concludes that "Bioremediation…will play an increasingly important role as a result of new and emerging techniques and processes." A specific finding pointed out in this report is that enzymes found in the fungus *Phanerochaete chrysosporium* or white rot fungus effectively degrade some wastes that prove resistant to most bacterial action (e.g. DDT and 2, 4, 5-trichlorophenyoxyacetic acid)."

Bonaventura et al, 2008 warn us, that "As the population of the planet continues to rise at alarming rates, natural checks and balances in the earths biochemistry may play more important roles, as waste materials like feces and urine begin to compete with and contaminate long standing human food sources." Is the day of the microbe upon us, or has it always been thus? The authors examine a collection of bioabsorption, biodegradation, and bioremediation processes before concluding generally that this emerging science provides a well demonstrated method for developing applied strategies in dealing with a wide spectra of ecologically degraded environments and areas that are at risk due to current and past activities of humans. No "proofs" are offered here, only a series of rational approaches to dealing with real problems that have potentially negative impacts on the human biosphere. In one of their summary statements, the may have said it best, "Bioremediation is a technological attempt to exploit the abilities of microbes and other members of the biosphere to restore and maintain environmental quality for all forms of life in the ecosystem, especially humans."

Skipper, et al, 1996 report on their attempt to "…document the existing status of the microbiology of environmental fate studies with pesticides." The authors assert that verification of data from laboratory studies to the field environment is needed, and that better field studies are also necessary to complete our understanding of the processes that ultimately breakdown and deposit these potentially lethal compounds. There is merit to this investigation, as the use of chemicals and pesticides seems to remain the preferred method in organized commercial farming and agriculture. These authors go into significant detail examining some of the more prevalent methods for determining the fate of pesticides as they move through their natural progression in the various environments of the world. There work seems through in its scope and critical examination, but in the end the conclusions they draw are somewhat banal and rather mainstream. They simply restate what we all knew, that the challenges here are complicated, and better practices need to come forth for deriving meaningful and accurate representation of the micro-compounds. Perhaps the "unknown" nature of their discussion can be seen as erring on the side of caution as we plan for current and so called sustainable forestry and agricultural processes in the future. That is, we should employ natural, benign processes whenever and wherever it is practical, to avoid adding fuel to the fire so to speak. If there is an implicit argument or finding to this study, perhaps this is it. Do not burn your maple leaves each fall because they make the sidewalk slippery, but mulch them lightly over your garden and lawn areas, as an alternative to dumping processed nitrates and the like into your rose beds?

In his recent book on mycoremediation, Singh (2006) notes that mycorrhizas provide a nutrient transport from soils to plant roots. Their role in the formation of soil aggregates and the protection of plants against drought and root pathogens is well established in the literature. These mediation processes occur over time, and depend on the successful development of a host root system associated with the fungal biomass. By selecting compatible host-fungus-substrate combinations these relationships can be exploited through careful study and inoculation of plant, riparian and other ecosystems. Future experiments should be designed to determine the precise fungal degradations in symbiotic field associations with a host plant or environment. Current knowledge on the functional diversity of mycorrhizal communities is fragmentary (Singh, 2006). Study of the effects of pollutants on the growth of extrametrical mycelium of these fungi is necessary in understanding their stability and sustainability in the ecosystems.

A large body of research is available that shows the potential for using mycorrhizal biomediation and bioremediation techniques in removing toxins from soils and groundwater, as well as in providing symbiotic and trophic benefits to a broad spectrum of environments and host organisms like trees, grasses, and riparian zones. This paper presents a sampling of this scientific data, but is not intended to be a complete listing of all the known works. The number is simply too large. In addition, there is clearly a gap between what is being suggested in the research, and the application of techniques in the real world that would benefit contaminated and otherwise degraded ecosystems.

Subsequent application of mushroom mycelia might employ naturally occurring plants that form beneficial symbiotic relationships in the environment, based on thorough study and organized planning methods. Lamar, et al (1999), have shown that a large variety of fungal species may be effective in remediating the presence of potentially harmful compounds in a variety of terrestrial and riparian zones including salmon spawning habitat locations. These include products like heavy metals, pesticides, petroleum, fuel oils, and others. The costs to introduce the methods should be fairly inexpensive and bio-friendly compared to practices like dredging, or introducing other chemicals that may be just as harmful. This author feels there is ample data in the research to suggest that mycorrhizal bioremediation techniques show considerable promise as biomediation factors." Considering the need to recover safe, healthy spawning and habitat areas for all fish species, further research on these processes may also eventually provide understanding of applied methods to reduce the presence of harmful substances in the food web for the entire planet. The potential benefits of this new branch of physical and organic science are enormous as we posture on the edge of an overgrown and polluted biosphere. The lowly mushroom is actually performing a multi-faceted role in its varied capacities as a primary decomposer of organics, a beneficial symbiot to other plants, an antibiotic toward harmful biota, an environmental architect at the biochemical level as it combines with and restructures toxic elements. Did we mention food sources and medicinal benefits? Is there a single organism, in its inherent complexity and pervasiveness that contributes as much to the sustainable architecture of the planet, and every carbon based life form it supports?

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