Best Mycorestoration Practices for Habitat Restoration of Small Land Parcels

by

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ABSTRACT

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La Dena Che' Stamets

Environmental pollution emanates from many sources causing harm to natural systems degrading human health. This thesis examines best *mycorestoration* (the use of fungi to prevent, reduce, repair, restore and ameliorate the negative impacts of chemical biological pollutants), practices, which provides low-cost, low-maintenance, time- conservative, effective biological solutions to remediate toxins. Throughout my extensive literature review mycorestoration practices demonstrated significant reductions in biological and chemical pollutants. The chemical toxins of focus were polycyclic aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's), chlorophenols, dioxins, DDT, trinitrotoluene and the hyper concentration-uptakes of heavy metals, including but not limited to: lead, uranium, arsenic from the environment. Fecal coliform bacteria **(**FCB) populations were effectively removed within ranges of 87 to 97%, whilst polycyclic aromatic hydrocarbons (PAHs) reductions ranged from 57 to 97% and total aromatic hydrocarbons (TAH) reductions were 91 to 99%, respectively

This thesis focuses on the three main pillars of mycorestoration: *mycoremediation, mycofiltration* and *mycoforestry*. I address the need and present an outline for an easy-to-use guide for mycorestoration projects that can be utilized at the grass-roots level. By healing polluted ecosystems with ecological restoration methods, this thesis reaches across multiple socioeconomic and environmental disciplines. Mycorestoration practices increase the inherent sustainability of human impacted habitats, reducing the need for remedial practices while fortifying the ecological services, which healthy habitats provide: clean water, clean food, clean air, and healthy inhabitants.

This thesis lays the foundation for future work and refinement of applications in this field. I suggest more development needs to address mycofiltration site design. The number of potentially useful species should be expanded, especially within white-rot fungi. Creating and compiling mycorestoration sites on the Geographic Information System (GIS) map would show locations of projects, including toxins identified and contamination levels before and after mycorestoration is applied. The implementation of standardization mycorestoration certification "stamp of approval" authorized by credible mycologists is a necessary first step before mycorestoration practices are deployed. Later work can focus on creating a multi-volume guide to Best Mycorestoration Practices specific to each eco-region to facilitate widespread pollution clean up.

List of Figures and Tables

Acronyms

Cd:	cadmium
COD:	Chemical Oxygen Demand
Cu:	copper
CWA:	Clean Water Act of 1972
DNR:	Department of Natural Resources
DOE:	Department of Ecology
DOH:	Department of Health
DNR:	Department of Natural Resources
DOT:	Department of Transportation
EcM:	ectomycorrhizal
EPA:	Environmental Protection Agency
ErM:	ericoid mycorrhizal
FLT:	flouranthene
NOAA:	National Oceanic and Atmospheric Administration
NPDES:	National Pollutant Discharge Elimination System
PAH:	polycyclic aromatic hydrocarbons
Pb:	lead
PCB:	polychlorinated biphenyls
PHE:	phenanthrene
POP:	persistent organophosphates
ppm:	parts per million
PYR:	pyrene
TAH:	total aromatic hydrocarbons

TKN:	Total Kjehldahl Nitrogen
TNT:	trinitortoluene
TPH:	Total Petroleum Hydrocarbon
TSS:	Total Suspended Solids
UO_3	uranium trioxide
U_3O_8	triuranium octaoxide
WADOH:	Department of Health
WADOT:	Department of Transportation
Zn:	zinc

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La Dena Che' Stamets, M.E.S.

Introduction

Environmental pollution emanates from many sources, causing harm to natural systems and degrading human health. Toxins include oil spills, excess nutrients from farm animals, and fertilizers affect terrestrial, riparian, and aquatic systems. Human health is closely linked to the health of these natural systems. Pollutants in our environment taint food supplies and compromise water quality, which in turn cause increases in the rates of cancer, asthma, learning disabilities, developmental impairment, and reproductive issues. For example, pesticides poison farms and wildlife while creating public health issues like birth defects and cancer (Amaranthus, 2009). Of the tens of thousands of chemicals currently used, few have been tested for their effects on human health. We know even less about combined effects of these toxic chemicals. This lack of knowledge leaves us unable to protect ourselves, let alone our children [[1]]. Because our country spends billions of dollars annually to treat illnesses caused by environmental pollutants protecting ourselves from toxins entering our environment should be a national priority.

The Washington State Department of Ecology (WSDOE) has two mandated ways to prevent toxic material from entering our soil and water. First, preventing use of toxic materials averts toxic exposures most effectively, avoiding future health and environmental costs. Second, the DOE assists businesses in reducing or managing the toxic chemicals that enter the environment. Should the toxins enter the environment, DOE will make an effort to clean up the polluted air, land, and water. The DOE's cleanup applications are necessary, but often incur extremely costly solutions.

One of DOE's main strategies is preventing toxic substances from entering

[1] http://www.ecy.wa.gov/toxics/index.htm

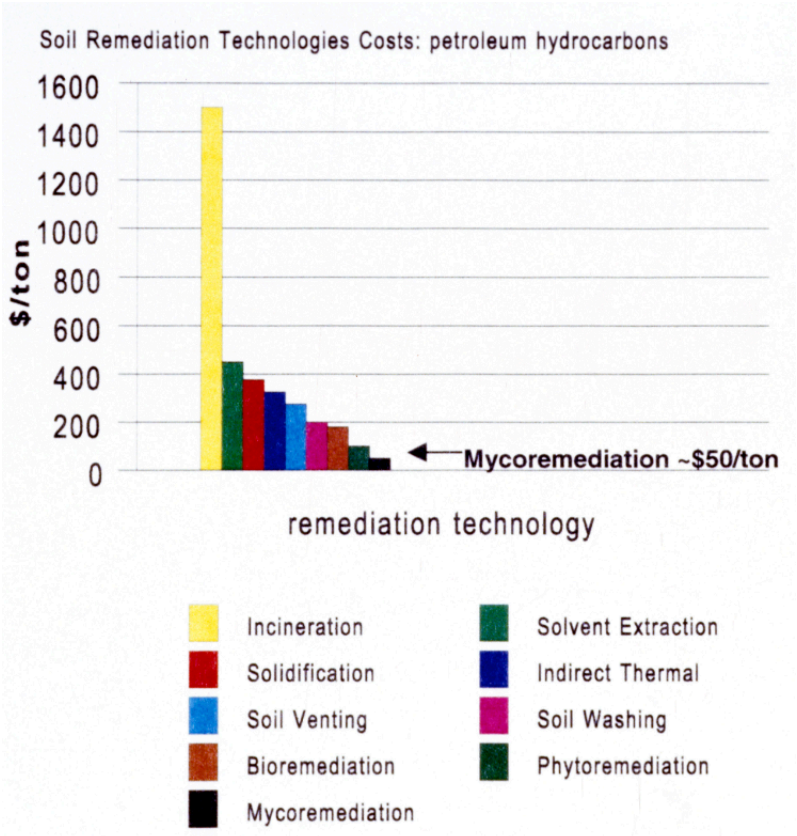
stormwater – rain and snow melt that runs off surfaces such as rooftops, paved streets, highways, parking lots, sidewalks, ditches and other vectors. As water runs off surfaces, it collects pollution such as oil, fertilizers, pesticides, soil, trash, animal waste, and "rubber" debris from tires. From these points of pollution, toxins travel down into aquatic systems (e.g., streams, rivers, bays, lakes, and oceans), or they may be detoured into a storm drain where toxic substances travel through storm pipes until the pollutants are eventually discharged, untreated, into our local water ways. A novel application – which is the basis of this thesis – is to explore the uses of fungal mycelium as a new strategy for dealing with multiple classes of pollutants that often converge as water carries them into downstream environments.

The study of fungi is known as mycology and a mycologist is a person who studies the kingdom of fungi. Mycorestoration is the use of fungi to prevent, reduce, repair, restore and ameliorate the negative impacts of chemical and biological pollutants. Myocrestoration methods can also restore and repair habitat whether damaged by human activity or natural disaster. Saprophytic, endophytic, mycorrhizal, and in some cases parasitic fungi can aid in this recovery. Saprophytic fungi grow upon dead organic material. Endophytic fungi join with living plants and the pairing is mutually beneficial, although each can live independently. Mycorrhizal fungi exist in an obligatory, symbiotic state. Typically, mushroom-forming mycelium grows on, or in, the roots of trees and other plants. Mycelium is the body of filamentous fungus; it is composed of a network of complexly branch hyphae (Trudell et al. 2009). The low-cost (see Figure 1: Soil Remediation Technologies Costs: Petroleum Hydrocarbons) of implementing

mycorestoration applications provides an attractive alternative biological solution compared to Best Management Practice (BMP).

Mycorestoration methods are a potentially low-cost, low-maintenance, biological solution to remediate toxins and heavy metals from the environment. Many stakeholders and government agencies, including the Washington State Department of Ecology (DOE) and the United States Environmental Protection Agency (EPA), may be well served by examining mycorestoration methods as a Best Management Practices (BMPs). In this thesis I address the need for an easy-to-use guide for mycorestoration projects that can be utilized at the grass-roots level. More specifically, my research targets individual landowners who own approximately 5-100 acres. Examples of some targeted properties include small-scale vehicle maintenance yards, agricultural enterprises, managed timberlands, livestock farms, and residential developments near watersheds or adjacent to aquatic systems (e.g., rivers, lakes, oceans, bays, creeks, wetlands, and tributaries). Mycorestoration applications can even be utilized to create buffers to protect endangered or keystone species from adjacent properties' contaminants. Though neighbors may not be consciousness about pollution leaching off their property, individual landowners can protect their investment by stopping the source of pollution before it negatively affects the habitat and health of adjacent lands.

Figure 1: *Soil Remediation Technologies Costs: Petroleum Hydrocarbons*



Note. *Table showing cost comparison of Polycyclic Aromatic Hydrocarbons (PAHs) remediation techniques.* Cost comparisons of remediation methods of polycyclic aromatic hydrocarbons. Created by Dr. Jack Word, formerly of PNNL/Battelle. (Paul Stamets 2006.)

By protecting our waterways from toxins and heavy metals, we secure economically significant industries like shellfish harvesting and fishing, which otherwise stand to suffer devastating economic losses. Polluted sites abound, and even though mycorestoration is not yet widely used, many parties express interest in small-scale projects. From the experiences of implementing small-scale projects, larger projects utilizing mycorestoration methods could become more practical. In addition, by focusing

on the Pacific Northwest (PNW) as a model, ecologically specific methods will be refined. Examining PNW pilot projects and case studies have shown positive results can be achieved by implementing these mycorestoration installations. Later the PNW model can be expanded to encompass other regions.

Motivating Factors: Mycorestoration in the Pacific Northwest of North America

How might individual landowners use mycorestoration to provide a biological solution to reduce toxins in both soil and water? This thesis constitutes a practical guide for individual landowners and fits into large-scale environmental and social contexts. Some aspects related to this topic cannot neatly be categorized into just environmental, or just social, contexts.

In an environmental context, my thesis topic addresses issues of anthropogenic toxins entering the environment, harming ecosystems on all three major habitat levels: terrestrial, aquatic, and riparian. The term "anthropogenic" refers to toxins or pollution caused by humans. Mycorestoration methods not only involve the study of mycology, but also have implications for the integration of hydrology, forestry, land-use planning, conservation, and human health.

In a social context, my thesis connects to political, economic, and historical issues. Through a political lens, my topic empowers individual landowners demonstrating that these biological solutions are not just for large-scale government applications. In fact these solutions can be implemented at the grass-roots level. From an economic perspective, mycorestoration installations cost less because they work with natural processes, and are less labor-intensive than other forms of BMP such as bioswales, sand filters, bioretentions cells (rain gardens) and fabric-filter membranes. BMPs are a compendium of proactive and often voluntary forest stewardship practices that have been determined to be the most effective, practical means of preventing or reducing soil and other pollutants from entering any water; streams ponds, lakes, wetlands, etc. Both fungi and bacteria naturally aid

wastewater treatment at an estimated ecological service value of $2.4 trillion annually (Primack, 2010).

The following example fits both into social and environmental contexts. For instance, excessive amounts of nutrients and toxins entering our environment hinder aquatic health making seafood unsafe for human consumption, causing both shellfish and fishing industries to stop harvesting and abate re-planting operations. This coincides with loss of revenue for employers, which reduces income for employees; the resultant negative economic consequences trickle throughout the entire larger economic system. This example has significant, but interrelated environmental and social dimensions.

The goal of small-scale mycorestoration projects such as those outlined within this thesis will enable and aid recovery of ecosystem health one installation at a time. If several small-scale mycorestoration projects reduce pollution at the input source or from adjacent properties the projects will reduce toxins and excessive nutrients, which locally concentrate. Ecological restoration is altering a site to reestablish – ideally – the original functioning ecosystem. By healing polluted ecosystems with ecological restoration methods, this thesis reaches across multiple socioeconomic and environmental disciplines.

The first motivating factor for remediation applications comes from individual landowners' realization that the environment health of their land and its surrounding areas is rapidly degrading. These immediate stakeholders see themselves as stewards of their own land. The motivation for creating this guide evolved from interest in mycorestoration projects and the potential for many small individual projects to have a cumulative positive effect on the entire environment. Stewards of the environment are looking for easy, low

cost, time- conservative, effective applications to reduce toxins and heavy metals that have negative impacts on both human and environmental health.

Within the Pacific Northwest (Fig. 2: Map of Pacific Northwest), the Puget Sound is the nation's second largest estuary reaching over 100 miles and including 19 river basins, in addition to meandering fresh-water and marine waterways. The Puget Sound connects to the ocean through the Straits of Juan de Fuca. Puget Sound is a small portion of what is known as the larger Salish Sea that is one of the most productive and populated estuary systems in the world.

Figure 2: *Map of Pacific Northwest*

Note. *Map of Pacific Northwest*
http://www.google.com/imgres?q=Pacific+Nw+Map+usgs

Rich in biodiversity, the Puget Sound supports 211 different fish species, 100 species of waterfowl, 26 species of marine mammals, more than several thousand species of fungi, in addition to thousands of invertebrates and plant species. Ecosystem diversity involves different biological communities and their associations with the chemical and physical environment. Moreover, ecosystem diversity is very rich in the Pacific Northwest, which classifies it as temperate rainforest (Benedict, 2011).

Archaeological records indicate that people have occupied the PNW since the end of the last glaciers' retreat; the Pleistocene era which was about 10,000 years ago (Goble, 1999). Another source states artifacts were found in the Puget Sound at two different archeological sites one, within the lower Columbia River and the other at the mouth of the Fraser River dating back 8,000 years ago (Kruckerberg, 1991).

Currently, surrounding the 2,500 miles shoreline, reside 4.4 million people, approximately 67% of Washington States' total human population. This includes 15 Native American Tribes (Makah, Nisqually, Puyallup, Quinault, Squaxin, Skokomish, Suquamish, Snoqualmie, Hoh, Quileute, Muckleshoot etc.) also known as First Peoples who rely on fishing and shellfish harvest as an economic industry and staple of their diet. These tribes are commonly referred as the Salish People. To these tribes, the Puget Sound is culturally and spiritually significant. They have relied on the natural resources of the Puget Sound for thousands of years. The linear extent of shoreline in the Puget Sound is comparable to the distance stretching from Washington State all the way to Washington D.C.!

Ice, water, and wind as well as earthquakes formed the PNW's unique landscape, and periodic volcanic rumblings each played a part in creating the Puget Sound and its

basin, which is commonly known as the Puget Sound Trough (Kruckeberg, 1995). The Puget Sound Trough reaches as far north as the artificial boundary of the United States and Canada, as far south as Olympia, Washington, as far east as the Cascade Mountains and as far west as the Olympic Mountains. Two major land shaping processes were the continental glaciations following the receding of the glaciers and the stream-cutting scouring channels which created the Puget Sound, Hood Cannel and Grays Harbor (Kruckeberg, 1995).

Large underwater formations keep most of the water in the Puget Sound circulating in the estuary. Approximately 150,000 pounds of untreated toxins go into the Puget Sound every day, threatening the health of this region's ecosystem. The Puget Sound Partnership has identified the main contributing factors to water pollution in the Puget Sound as human waste, stormwater and industrial discharge. One third of all shellfish beds show evidence of fecal coliform bacteria contamination in Washington State (Washington State Department of Health (WSDOH). Furthermore, the US EPA found 30,000 acres of commercial shellfish beds have been forced to close over the last 25 years because of bacterial pollution.

This pollution impacts human health, causing cancer and birth defects and reaching across all spectra of income. Within the United States, over 50 % of the nation's drinking water wells contain measurable amounts of nitrate and 7 % have detectable amounts of pesticides. In the U.S. alone approximately 12 billion dollars annually are spent on health and environmental costs associated with pesticide usage, while estimated yearly public and environmental health costs related to soil erosion is 45 billion dollars. If this continues future generations might not be able to produce food for basic survival

(Amaranthus, 2009).

The scope of this thesis is limited to the most widespread toxins; among these are polyaromatic hydrocarbons (PAHs), heavy metals, and fecal coliform bacteria (*Escherichia coli*). These toxins are widespread and have extremely negative long-term effects on environmental health (EPA).

For simplicity I have organized this guide into two parts. Part One explains the different applications of mycorestoration along with case studies and pilot projects. The purpose of this section is to demonstrate the methods' effectiveness to potential users. Case studies are distinguished from pilot projects in that they are completed studies with reported results. Pilot projects, on the other hand, are promising ongoing projects where results are not yet available. I will define three major categories of mycorestoration, which I call "Pillar I, II, and III": Mycoremediation, Mycofiltration and Mycoforestry. Pillar IV, Mycopesticide is still highly experimental, and so is not addressed in this thesis which presents only well understood best mycorestoration practices. I will go through each of the three pillars in detail, offering for each several scientific case studies and pilot projects. Part Two focuses on methodology for grass-roots implementation.

1. **Mycoremediation** centers on the use of fungal mycelium to degrade pollutants *in-situ* (i.e., at the place where the original pollution occurred). An example would be ameliorating an oil spill on land by mixing or layering mycelium onto the polluted soil. Mycoremediation is a biological solution where saprophytic fungi are used to decompose toxins in the environment. Saprophytic fungi digest dead organic matter whereas mycorrhizal fungi live in a symbiotic relationship to most plants and trees. Saprophytic and mycorrhizal fungi combine to improve plants' water uptake, eliminate pathogens, and

make plants' natural defenses stronger. Mycoremediation has been known to metabolize petroleum hydrocarbons, and to capture and immobilize heavy metals such as lead, uranium, and mercury. Fungi have also been known to metabolize chemical pollutants including, but not limited to: chlorine, dioxins, persistent organophosphates (POPs), polycyclic aromatic hydrocarbons (PAHs), total aromatic hydrocarbons (TAHs), polychlorinated biphenyls (PCBs), and trinitortoluene (TNT).

2. **Mycofiltration** is the use of mycelium to capture and ameliorate flowing chemical and biological pollutants, thus preventing them from entering sensitive downstream habitats. Stamets (2006) describes mycofiltration as the use of fungi as a membrane for filtering out microorganisms, pollutants and silt. Habitats infused with mycelium reduce downstream particulate flow, mitigate erosion, filter out bacteria and protozoa, and modulate water flow through the soil. Chemical pollutant examples include, but are not limited to: chlorine, dioxins, persistent organophosphates (POPs), polycyclic aromatic hydrocarbons (PAHs), total aromatic hydrocarbons (TAHs), polychlorinated biphenyls (PCBs), and trinitortoluene (TNT). Examples of biological pollutants include bacterially rich runoffs. An example of mycofiltration would be placing mycelium mycofilters below a livestock farm, which captures fecal coliform bacteria and excessive nitrogen runoff, thus preventing harmful algal blooms that hinder shellfish harvesting and fishing industries.

3. **Mycoforestry** is the use of fungi beneficial to trees to aid the regeneration of forests. For instance, the establishment of a new forest on land devastated by repetitive slash-and-burn clear cutting practices could be regenerated by mycoforestry. Mycorrhizae have a

symbiotic relationship with 90% of the plants on earth (Amaranthus, 2009; Primack, 2010). Mycorrhizae have a close, long term biological relationship with another plant or tree, and the two species are generally found living concurrently in beneficial symbiosis, evolving and coexisting together. The plant or tree gives the mycorrhizae sugars and in exchange the plant is fed minerals and is protected from pathogens, thus increasing uptake in water and experiencing greater growth as compared to plants without mycorrhizae. Mycorrhizae can scarify themselves in a time of drought, to save host plants, and re-generate as the plants recover.

At the end of each section, after explaining the three main pillars of mycorestoration, a critique is given.

Part Two focuses on methodology for grass-roots implementation of small-scale projects. Low and high tech methods are both given. Lastly, I will offer my conclusions and suggestions for future research in the emerging field of myco-ecological science. If pollution sources remain unaltered, pollution continues to compound. Having information for small-scale mycorestoration projects will help individuals address pollution issues on their property.

Part I

Mycorestoration

Mycorestoration is an umbrella term for using mushroom mycelia – fungal networks of thread like cells or hyphae – as a biological platform for cleaning up toxins and heavy metals in the environment. Because it's a biological solution, each mycorestoration project must be designed specifically for a particular site, commonly referred to as "site-specificity". Which kind of mycorestoration is best for your land (mycoremediation, mycofiltration or mycoforestry) depends on the landscape you are remediating. All three applications involve the following tasks:

1) Analysis of habitat/inventory of the site needs to be conducted.
2) Toxins, heavy metals, native fungi, plants and animals (both terrestrial and aquatic) in the studied environment should be determined through sample testing.
3) Once tasks (1) and (2) have been accomplished, bench and mesocosm field application studies are conducted to see if mycorestoration will likely be effective at removing toxins or heavy metals from the landscape.
4) Finally, field application is executed at a seasonally appropriate time, commonly during the spring in the PNW. A spring execution allows for the first flush (i.e., fruiting) of mushrooms in following fall.
5) Several months after (4), the extent to which the mycorestoration was effective in reducing pollutants in contaminated soil or water should be determined. Soil and water samples are taken both above and below the installation, and subsequently analyzed.

How do toxins enter our environment? They enter into our environment via a variety of different anthropogenic vectors: pesticides, herbicides, fertilizers, munitions, textile dyes, wastewater treatment plants, illegal dumping and estrogen-based pharmaceuticals, to name a few. Fig. 3: Toxins and Their Primary Origins shows where these contaminants came from. All these aforementioned industrial activities and products contribute to and are susceptible to being broken down by mycelium enzymes. Some species of fungi have proven to be more effective than others (Stamets 2006).

Figure 3: *Toxins and Their Primary Origins*

Toxins, Their Primary Origins and Research Showing Efficacy of Their Fungal Degradation

Type of Toxin	Products or Processes That Emit Toxins	Supporting Research References
Anthracenes	Dyes, pesticides, and derivatives: benzo(a)pyrenes, wood preservatives, fluorene, naphthalene, acenaphthene, acenaphthylene, pyrenes, biphenylene	Johannes et al. 1996; Knapp et al. 2001
Anthraquinones	Dyes	Kasinath et al. 2003; Minussi et al. 2001; Novotny et al. 2001, 2003; Hatvani and Mecs 2003
Benzopyrenes (PAHs)	Incinerators	Qiu and McFarland 1991
Chlorinated aromatic compounds: pentachlorophenol (PCP), trichloro-phenol (TCP), polychlorinated biphenyls (PCBs), dioxins, chlorobenzenes	Transformers, lighting fixtures, paper products, chlorine bleaching, paints and coatings	Gadd 2001
Copper/chromium	Treated wood	Humar et al. 2004; Illman et al. 2003
Dimethyl methylphosphonates (DMMP)	Chemical warfare agents: VX, sarin, soman	Thomas et al. 1999; Word et al. 1997
Dioxins	Incineration of industrial wastes, forest fires/wood burning, coal-fired plants	Chiu et al. 1998
Pentachlorophenol	Pesticides, preservatives	Kondo et al. 2003
Pesticides	Alachlor, aldrin, chlordane, DDT, hep-tachlor, lindane, mirex, atrazine, benomyl	Gadd 2004
Petroleum hydrocarbons	Oil, coal, tar, gasoline, diesel	Bhatt et al. 2002; Cajthaml et al. 2002; Eggen and Sasek 2002; Sasek 2003; Thomas et al. 1999; Moder et al. 2002

Not all mushroom species break down toxins and bioaccumulation of heavy metals. Mushroom species are classified into two fungal subcategories: brown rotters and white rotters. The focus on hydrocarbon degradation has been to use white rotting fungi. The manganese-dependent peroxidase enzyme produced by white rot mushrooms breaks down the wood and bonds of hydrocarbons. Bonds in petroleum products share a similar type of bond that keeps the backbone of woody plants – the lignin– together. Moreover, this manganese-dependent peroxidase enzyme can be used to decompose a variety of hydrocarbons: e.g., different oils, diesel, pesticides, and herbicides. After hydrogen-carbon

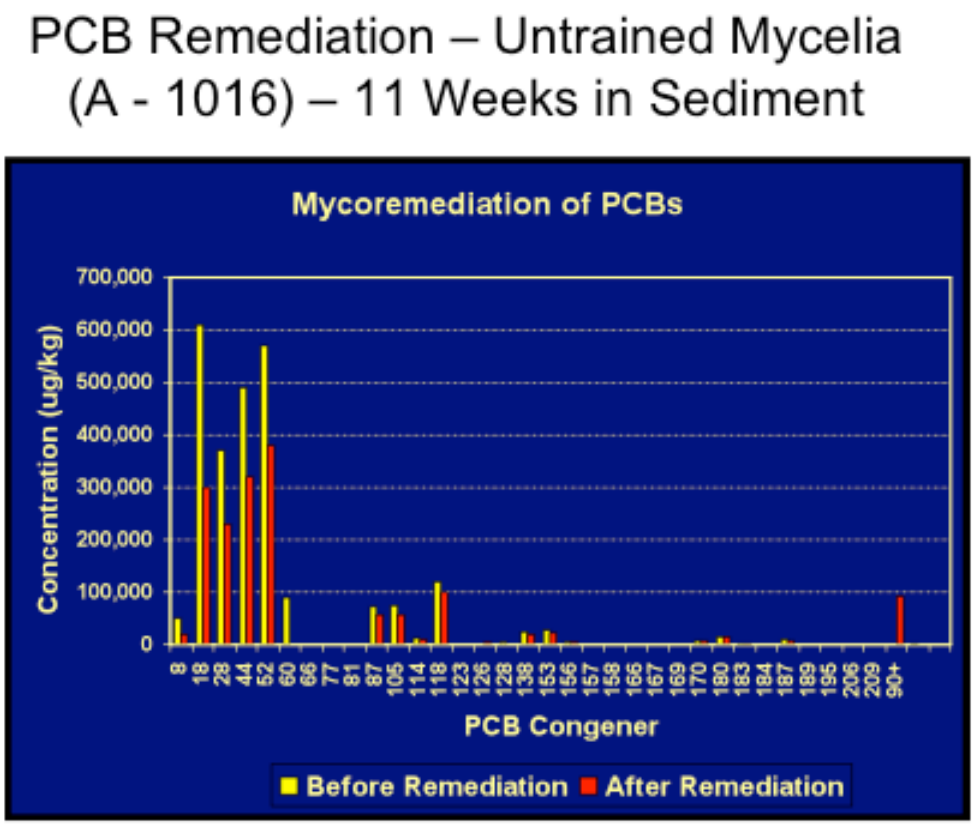
bonds are broken by mycelium, the leftover non-solid byproducts are primarily water and carbon dioxide. Once the remediation is completed the organic material loses volumetric mass, 50% is liberated as gaseous carbon dioxide and a 10-20% is converted into water (Stamets, 2006).

How do white rotters achieve remediation? White rotters create a ligninase enzyme that metabolizes brown fiber in wood but leaves the cellulose structure behind, causing the wood to have a whitish appearance (Cabello 2001, Sasek 2003, Stamets 2006). White rotters break down toxins through a similar process as the metabolization of wood fiber. A substrate is rendered light in color from the fungal decomposition of lignin (delignification), leaving cellulose largely intact. Solid blocks of wood can be utilized for testing whether fungus causes white rot or brown rot. Most brown rotters, however, are not as effective in bioremediation of a wide array of toxins typical as are white rotters. White rotters are also more plentiful in the forest than are brown rotters. White rot fungi have the fastest degrading rates. White rotter species examples are: the oyster mushrooms (*Pleurotus ostreatus* and other Pleurotus species, and subspecies), maitake (*Grifola frondosa*), turkey tails (*Trametes versicolor* and Trametes species), reishi (*Ganoderma lucidum*), artist conk (*Ganoderma applanatum*), and crust fungi (*Phanerochaete chrysosporium*). All these species have been proven to degrade POP's and PAHs. This is important because white rot mushrooms are mycoremediators of toxins that are joined together by hydrogen-carbon bonds (Stamets 2006, Thomas, 1999).

Mycoremediation and mycofiltration can capture and metabolize the flow of toxins such as fecal coliform bacteria (found in wastewater from farming practices or failing septic systems), organophosphates (found in pesticides, detergents, and fertilizers), and

PCBs (e.g., those found in insulating fluids within electrical equipment in power plants, industries, and large buildings). See Fig. 4: PCB Remediation- untrained mycelia. Specific mushroom-forming fungi (Basidiomycetes) have been shown to break down certain toxins more effectively than others. Earlier research by Adinarayana et al. (2001) found in both laboratory and field scale studies that fungal inoculants successfully detoxified persistent organophosphates. This process of reducing the toxicities produces a less harmful compound than other methods that can be naturally absorbed within the environment. Future studies are needed to focus on analyzing the wide array of fungal species and their effects on different chemical contaminants (Adinarayana, 2001), and it is clear that making use of fungi is a challenging, important task that could lead to useful byproducts, saving energy and preventing pollution (Cohen, 2001).

Figure 4: *Chart of polychlorinated* biphenyls *(PCBs) Remediation of Untrained Mycelia after 11 weeks in sediment.*



Note. This chart illustrates reduction in PCBs concentration within soil after 11 weeks of mycoremediation treatment, data derived from Jack Word, New Fields Washington personal communication project.

Mycologist Paul Stamets has identified which fungal species influence specific contaminants. See (Fig. 5: Mushroom with Activity Against Chemical Toxins & 6: Mushroom Species effects on Heavy Metals) and Fig. 7 for photos of mature fungal fruit-bodies commonly used in mycorestoration installations.

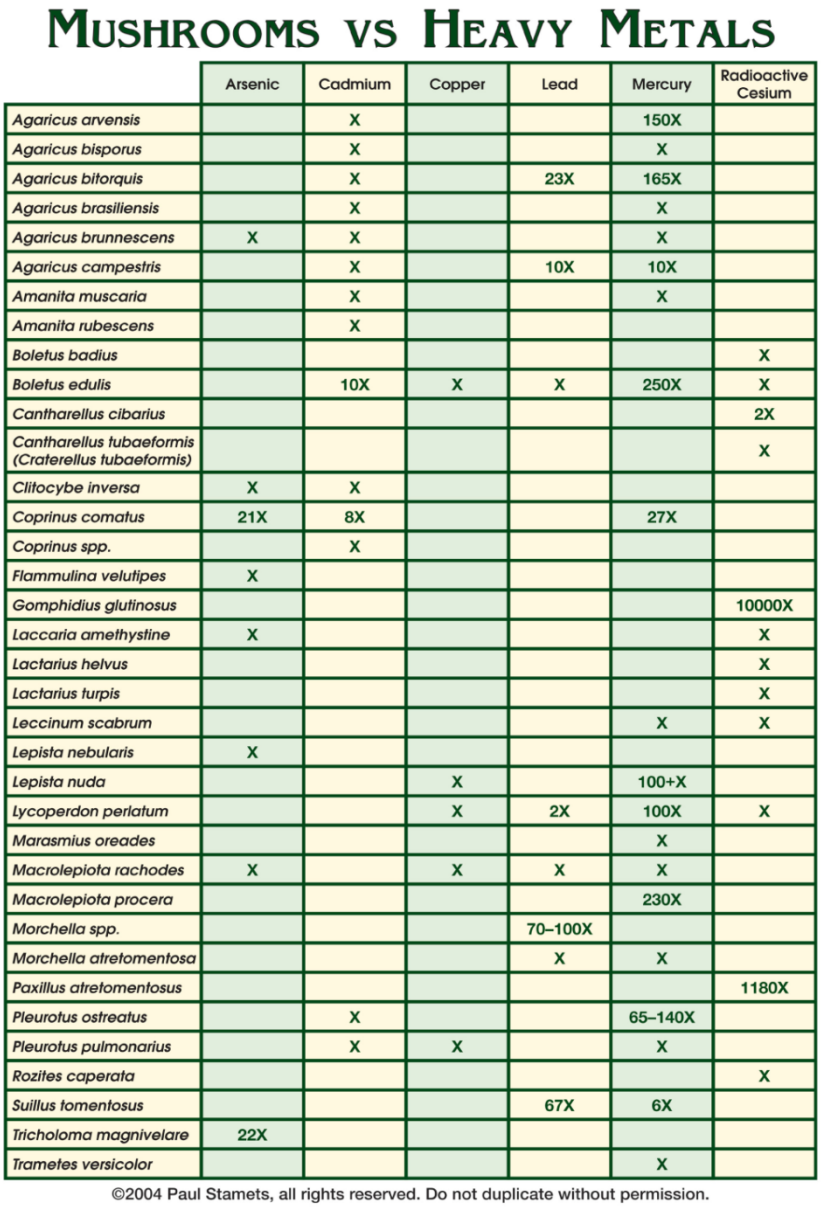
Figure 5: *Mushroom with Activity Against Chemical Toxins*

Mushrooms with Activity Against Chemical Toxins

	Anthracenes	Benzopyrenes	Chromated Copper Arsenate	Chlorine	Dimethylmethylphosphonate (VX, Soman, Sarin)	Dioxin	Persistent Organophosphates (POPs)	Polycyclic Aromatic Hydrocarbons (PAHs)	Polychlorinated Biphenyls (PCBs)	Pentachlorphenols (PENTAs)	Trinitrotoluene (TNT)	Brown (B) or White (W) Rot?
Antrodia radiculosa			X							X		B
Armillaria ostoyae					X							W
Bjerkandera adusta		X						X				W
Gloeophyllum trabeum			X			X						B
Grifola frondosa									X			W
Irpex lacteus								X				W
Lentinula edodes								X	X	X		W
Meruliporea incrassata			X							X		W
Mycena alcalina				X								?
Naematoloma frowardii (=Hypholoma)								X			X	W
Phanerochaete chrysosporium		X								X	X	W
Pleurotus eryngii						X						W
Pleurotus ostreatus		X			X	X		X	X		X	W
Pleurotus pulmonarius						X					X	W
Psilocybe spp.					X		X					W
Serpula lacrymans			X					X				B
Stropharia coronilla												W
Trametes hirsuta										X		W
Trametes versicolor	X		X		X	X	X			X	X	W

Note. This chart shows Mushrooms species that are able metabolize chemical toxins.

Figure 6: *Mushroom Species' concentrations of Heavy Metals* (Stamets 2006)



MUSHROOMS VS HEAVY METALS

	Arsenic	Cadmium	Copper	Lead	Mercury	Radioactive Cesium
Agaricus arvensis		X			150X	
Agaricus bisporus		X			X	
Agaricus bitorquis		X		23X	165X	
Agaricus brasiliensis		X			X	
Agaricus brunnescens	X	X			X	
Agaricus campestris		X		10X	10X	
Amanita muscaria		X			X	
Amanita rubescens		X				
Boletus badius						X
Boletus edulis		10X	X	X	250X	X
Cantharellus cibarius						2X
Cantharellus tubaeformis (Craterellus tubaeformis)						X
Clitocybe inversa	X	X				
Coprinus comatus	21X	8X			27X	
Coprinus spp.		X				
Flammulina velutipes	X					
Gomphidius glutinosus						10000X
Laccaria amethystine	X					X
Lactarius helvus						X
Lactarius turpis						X
Leccinum scabrum					X	X
Lepista nebularis	X					
Lepista nuda			X		100+X	
Lycoperdon perlatum			X	2X	100X	X
Marasmius oreades					X	
Macrolepiota rachodes	X		X	X	X	
Macrolepiota procera					230X	
Morchella spp.				70–100X		
Morchella atretomentosa				X	X	
Paxillus atretomentosus						1180X
Pleurotus ostreatus		X			65–140X	
Pleurotus pulmonarius		X	X		X	
Rozites caperata						X
Suillus tomentosus				67X	6X	
Tricholoma magnivelare	22X					
Trametes versicolor					X	

Note. This chart displays different species of fungi against different heavy and their ability to mobilize heavy metals.

Figure 7: *Mature Fungal Fruit-bodies Commonly Used in Mycorestoration*

Note. From left to right; *Pleurotus ostreatus* (Pearl Oyster), *Stropharia rugosoannulata* (King Storphraia), and *Tremetes versicolor* (Turkey Tail). Photographs by: La Dena Che' Stamets 5-18-2012.

The ability of fungi to influence contaminates is generally attributed to the lignin-degrading enzyme system of the fungus. Within soil conditions, fungal degradation capabilities are affected by processes similar to bioremediation such as bioavailability, temperature and other physical parameters, and pollutant toxicity. Optimal performance of white-rot fungi introduced into the soil depends on its survival, soil matrix, and relation to autochthonous soil micro flora. Filamentous fungi grow hyphae that invade soil substrates, secreting water-laden enzymes that degrade polymeric matter that is then utilized as nutrients by other plants. Further, fungi are highly adaptive can grow with low moisture, and many species are valued for human consumption.

The development of fungal technologies has been hindered because scientists initially have generalized to all white-rot fungi results derived only from *Phanerochaete chrysosporium.* The physiological and ecological diversity of other white-rot fungi warranted further investigations. (Sasek, 2003). Cerniglia (2001) observes that numerous experiments have proven that white rot mushrooms can remove PAHs and other complex mixtures. Results have been positive in laboratory tests as well as in petroleum contaminated soils: "… the biotransformation process may be characterized as a sequestration that can lead to eventual detoxification" (Cerniglia, 2001).

Pillar I: Mycoremediation

Mycoremediation has held promise for cleaning up polluted soils since 1985 when it was discovered that white rot fungus *Phanerochaete chrysosporium* could metabolize a number of important environmental pollutants, including polycylic aromatic hydrocarbons (PAH's), polychlorinated biphenyls (PCB's), chlorophenols, dioxins, DDT, trinitrotoluene, and synthetic dyes (Eggen et al. 2002, Sasek et al. 2003). This ability to degrade chemical pollutants of different compounds is caused by the extracellular enzyme system (lignin peroxidase, manganese-dependent peroxidase and laccase) and production of free radicals (Eggen et al. 2002, Stamets 2005). Mycoremediation is a type of bioremediation that utilizes fungal enzymes' natural ability to break down anthropogenic contaminants, allowing ecological succession to take place naturally. Succession is the gradual replacement of one group of organisms by another over time following initial disturbance. This section will describe the science behind the application of mycoremediation for restoring contaminated soil.

Soil contaminated by PAHs is well documented; the persistence and compounding of PAHs are tantamount to significant ecological risk because of toxicity, potential carcinogenicity, and resistance to bioremediation. Therefore, these compounds are on the US EPA's Priority Pollutant list, which includes 129 different pollutants. Below I describe mycoremediation of soils contaminated with PAHs.

Using experiments with fungi and yeasts naturally found in aquatic sediment, surface waters, and terrestrial environments, Cabello (2001) found that fungi and yeasts can be used to biodegrade PAHs. Fungi can penetrate the soil whereas bacteria cannot; fungi accomplish this penetration by sending out infiltrating fungal hyphae that exude enzymes which reach subsoil PAHs. Other lignin-degrading fungi enzymes can decompose wood and break down and degrade a variety of toxins (Cabello, 2001).

Eggen and Sasek (2002) describe the capacity of spent substrate from commercial mushroom production of *Pleurotus ostreatus* to remove PAHs from weathered creosote in highly contaminated soil from an abandoned wood preservation site. Adding the spent fungal compost resulted in reductions from 50% (acenaphthene, anthracene) to 87% (phenanthrene, flourene) within a twelve-week treatment. The reduction increased to 87% (anthracene) and 97-99% (phenanthrene, flourene, acenaphthene) after additional re-inoculation with fungal substrate and an added three-week incubation period. After a twelve-week fungal treatment period flouranthene and pyrene decreased by approximately 43% and 34%, respectively.

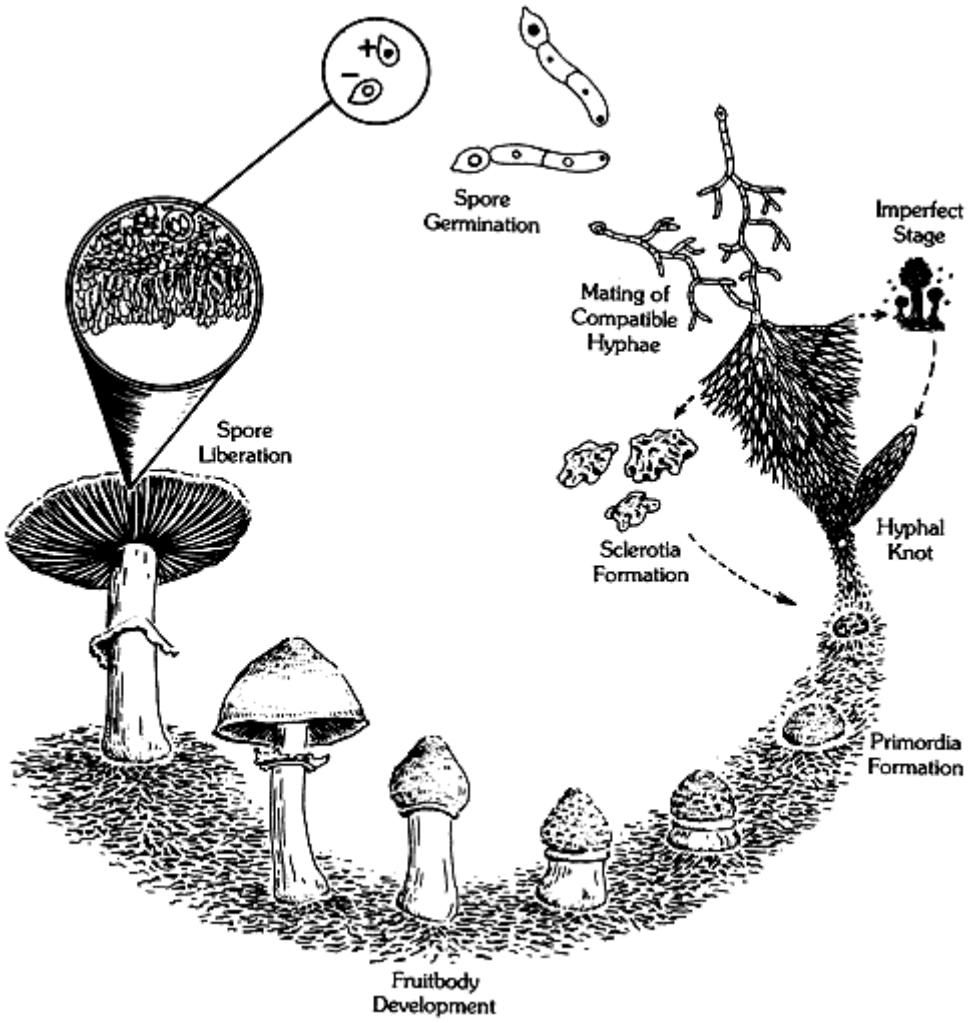
Spent compost of *Agaricus bisporus*, commonly known as white button mushroom, has been utilized by gardeners to fertilize and condition soils on disturbed commercial sites. Several species of fungi (*Pleurotus ostreatus, Trametes versicolor,*

Agrocybe aegerita, Kuehneromyces mutabilis and *Stropharia rugosoannulata*), are edible and medicinal, commonly used to benefit human health, and have been extensively studied (Eggen et al. 2002). These also enable environmental health by metabolizing chemical toxins.

After eight weeks of fungal treatment the average degradation was ~ 40% and after a 16-week period, scientists observed that degradation rates had reached 80%. After fourteen-months of fungal treatment the residual hydrocarbon concentrations were approximately 7% of the original level (Eggen et al. 2002).

The success in using fungi for bioremediation is largely determined by which fungi are selected, and how they interact to abiotic (non-living) and biotic (living) systems. In addition, the remediation application also needs to take into account the particular natural biological cycle of the fungi selected. See Fig. 8-Chart3- *The mushroom life cycle*. Fungi selected must be able to compete with native soil bacteria because different fungi and bacteria either work together to destroy the PAHs or compete and thus destroy each other. One must carefully consider how nutritional factors in soils and environmental factors influence biodegradation rates. Cerniglia (2001) pointed out that a successful mycoremediation method will also be reliable enough to meet government regulatory requirements. Methods for the detoxification of PAH residues in the environment must be cost-effective, quick and environmentally safe.

Figure 8: *The mushroom life cycle*



Note. This illustration shows the Mushroom Life Cycle. Hyphae are long tube-like elements that make up the body (mycelium) of a fungus, and may or may not be separate. Starting at spore liberation to spore germination to growing hyphae, creating hyphal knots (and sometimes sclerotia formation), followed by primordial formation fruitbody developes which then matures to what we see as a harvestable mushroom. Spores liberated from the mushroom begin the life cycle anew. Climatic conditions may both limit and aid growth of cycle. © www.fungi.com

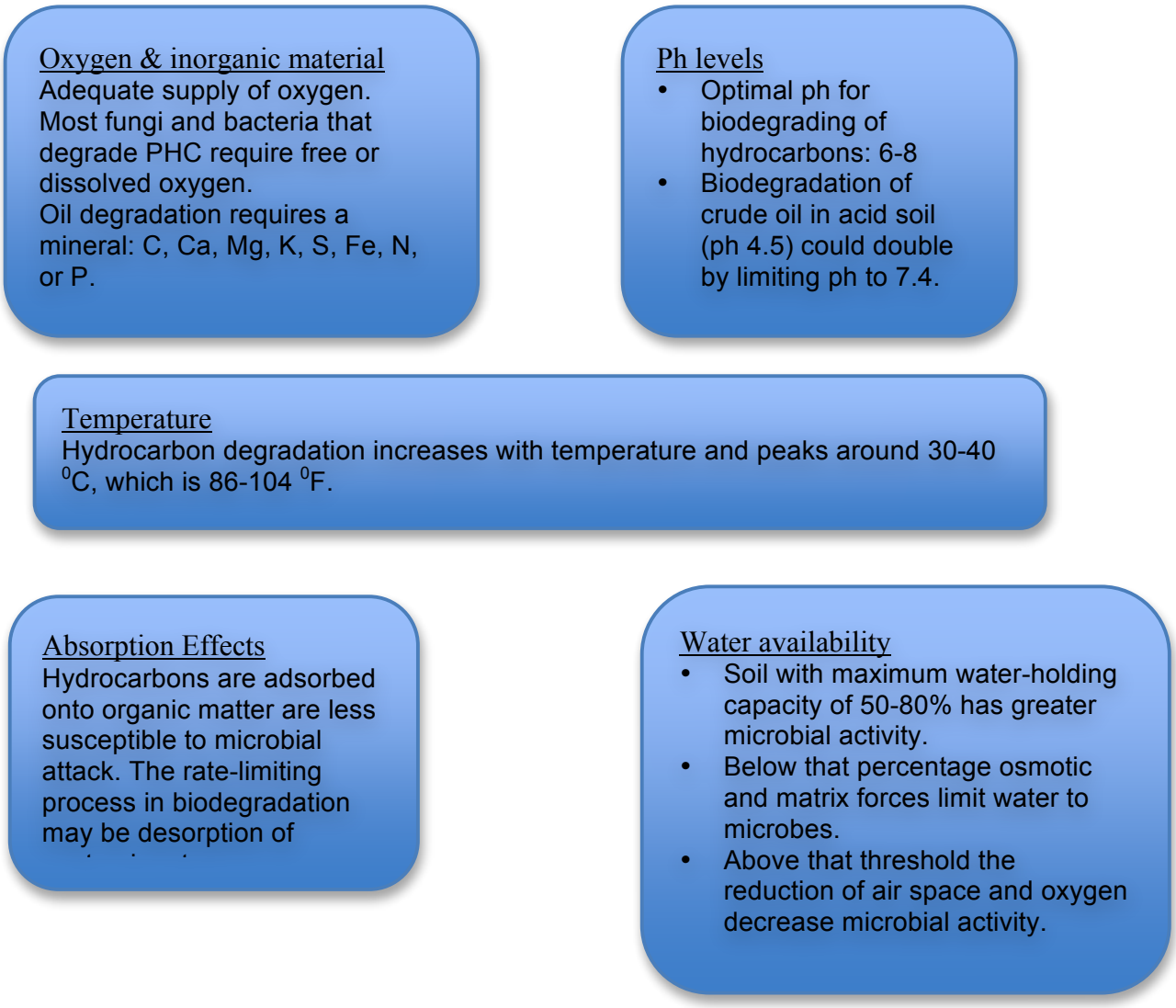
Anyone utilizing mycoremediation should first try native mushrooms specific to that location. If local mushrooms cannot be reproduced in the lab or there is no evidence of mushrooms in that particular location, the next step is to use Pearl Oyster Mushrooms *(Pleurotus ostreatus)* local to that bioregion, if not specifically to the toxic waste site. Certainly, there is no threat of species becoming problematic because the saprophytic mushrooms will run their natural life cycle and then die off after substrate decomposition (Fig. xx). Instead of a black, toxic, smelly, lifeless pile of soil, decomposition creates an oasis for other species such as insect larvae, plants, and birds to inhabitant the area (Stamets 2006, WSDOT 1998, Battelle 1999).

What is crude oil? Crude oil is complex mixture of hydrocarbons and non-hydrocarbons that are toxic to living systems. It is used for energy and raw material for industries. Increased demand for energy results in increased production, transportation and refining of crude oil, which results in increased pollution of the environment. The main source of petroleum hydrocarbon pollution in the environment is low-level discharge, e.g., urban run-off, cleaning operations, and oil treatment. These non-point sources of pollution combined together account for 90% of the total anthropogenic petroleum pollution. Other oil pollution comes from oil-well blowouts, seepage, and de-ballasting, sale and usage of petroleum products, pipeline overflow and breakage, and storage tank spills (Obire, 2003). Obire reported deliberate discharge of oil field wastewater or effluent (liquid waste or sewage discharge into a river or the sea) as a source of environmental contaminants. Obire et al. (2009) later assessed the sources of crude oil pollution and its effects on the environment and microorganisms. Subsequently, they compared methods using many

different species of fungi that are occur in oil-polluted environments and were known to degrade PAHs.

The level of toxicity of petroleum products and crude oil varies based on composition and concentrations, biological state, environmental factors like weathering, and the biological state of the organism when contamination occurs. See Fig 9: *Parameters of Bioremediation Process (Created by LaDena Stamets, reference Obrie 2009).*

Figure 9: *Parameters of Bioremediation Process*



Note. Created by LaDena Stamets. Data derived from the following references: Obire et al., Fungi in Bioremediation of Oil Polluted Environments 2009.

In heavily polluted areas the effects are instantaneous and detrimental to the ecosystem and its inhabitants, including plants, animals life, and agriculture. Species at different stages in their life cycles will have different susceptibilities to pollution (Obire, 2009). Fungi are amenable to large-scale production, efficiency, genetic engineering or manipulation, cost effectiveness, and ease of transportation. Certain fungi are known to

possess crude oil biodegradation potential. Besides the classic mushroom-forming fungi like *Pleurotus ostreatus*, many other non-mushroom forming fungi have been proven effective in digesting hydrogen, and include but are not limited to the following twenty-four genera hosting species proven potentially useful for mycoremediation:

Acermonium
Aspergillus
Aureobasidium
Candida
Cephalosporium
Cladosporium
Cunninghamella
Fusarium
Geotrichum
Giiocladium
Sporobolomyces
Torulopsis
Graphium
Hansenula
Mortierella
Mucor
Paecilomyces
Penicillium
Rhodosporidium
Rhodotorula
Saccharomyces
Sphaeropsidales
Trichoderma
Trichosporon

Obire et al. (2009) argue that fungi might have an important role in oil cleanup within the Niger Delta but further studies are needed to apply those techniques in the region. Obire concludes fungal mycelia are can penetrate oil and increase surface area that can then be degraded by other microbes. Fungi have the ability to grow under harsh environmental conditions, for instance, where low pH and poor nutrient limit bacterial growth. Moreover, fungi are easy to transport, genetically engineer and can be multiplied

into large quantities. Thus, Niger Delta would benefit from this biological technology to clean up its oil-polluted environment.

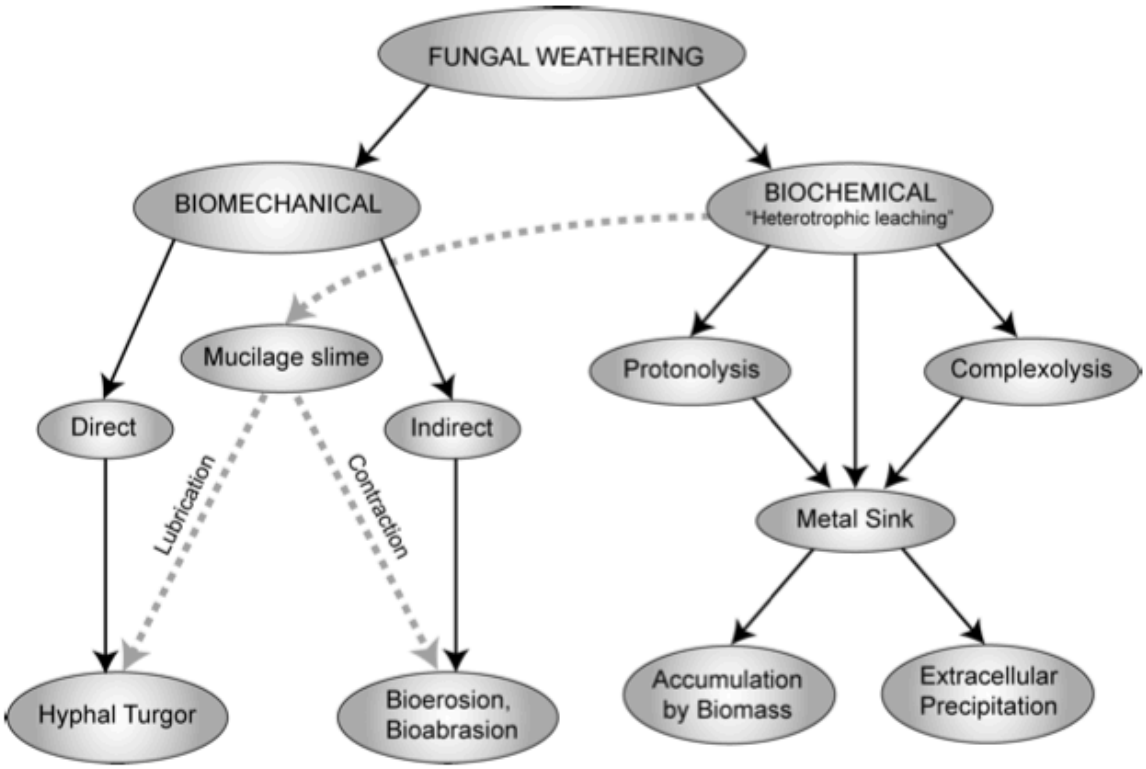
Heavy metals are of equal concern to environmental pollution from petroleum products. Although depleted uranium is less radioactive than natural uranium it has the same chemotoxicity and is harmful to human health. Uranium is utilized in nuclear power plants to generate the heat in reactors, and produces material for nuclear weapons. Uranium is also used as a pigment to color glass to produce orange, yellow, and red hues and was also used for tinting and shading in early photography.

Lead enters our environment in many ways: lead shots from hunting, fishing weights or jigs, and industrial waste (e.g., paints printing inks, lead water pipes, lead glazed pottery, battery casings). Lead in all of its forms is a potentially dangerous pollutant because of its toxicological effects on humans. In the human food-web toxins including mercury, lead, dioxins and polychorobiphenyls (PCBs) are passed on throughout each trophic level becoming more concentrated, in a fashion similar to how toxicity concentrates in harmful algal blooms (HAB) that are lethal to secondary consumers. HAB can lead to eutrophication; the process of degradation in aquatic environments caused by nitrogen and phosphorus pollution, characterized by algal blooms and oxygen depletion. Biomagnification is the process whereby toxins become more concentrated in animals that are at the higher levels in the food chain. Organisms that are primary producers include green plants, alga, or seaweed and obtain their energy directly from the sun via photosynthesis; such organisms are also known as an autotrophic or photosynthetic species. Levels of biological communities representing ways in which energy is captured and moved through the ecosystem by the various types of species is referred to as trophic

levels. Primary producers are herbivores, secondary consumers are carnivores or detritivores. Mammals at the top of the food chain experience concentrated levels of toxins through digestion, resulting in chronic diseases such as neuropathy and cancer. Mycelia can break down these toxins in soil before they enter our food supply. Furthermore, saprophytic fungi live on dead organic matter and are among the first organisms to rejuvenate the food chain after catastrophes (Stamets, 2006).

Heavy Metal Mobilization. The accumulation of heavy metals in soil and water causes harm to humans because we bioaccumulate heavy metals through consumption. Geomycology is the scientific study of the roles of fungi in processes of fundamental importance to geology, and the biogeochemical importance of fungi is significant in several key areas. These include nutrient and element cycling, rock and mineral transformation, bioweathering, mycogenic biomineral formation, and interactions of fungi with clay minerals and metals. These processes can occur in aquatic and terrestrial environments, but it is within the terrestrial environment that fungi are thought to be the greatest effective geochemical influence (Gadd, 2011). Geoffrey Gadd and his colleagues have conducted extensive scientific research on this topic by using fungi to mobilize toxic metals, lead, and uranium. Please refer to Gadd's flowchart (fig. 10: *Mechanisms of fungal bioweathering of mineral surfaces applicable to myco-deterioration of concrete)* for clarification.

Figure 10: *Mechanisms of fungal bioweathering of mineral surfaces applicable to myco-deterioration of concrete*



Note. Mechanisms of fungal bioweathering of mineral surfaces applicable to myco-deterioration of concrete. Gadd et al., (2007) Fungal Degradation of Barrier Concrete used in Nuclear Waste Disposal.

Gadd has extensively explored the ability of fungal species to uptake heavy metals, and explains how fungi aid metal transformation: "Mobile metals can be either be bound, accumulated or precipitated by fungal biomass through biosorption to biomass" (Gadd et al. 2007). Immobilization can result from bio-absorption. The fungi can take up metal cations into forms that can be made available intracellularly and incorporated into biogeochemical processes. Although bioabsorption is a relatively new concept, it's a promising field. In addition to revealing scientific insight into metal bioabsorption, Gadd deepens our understanding of how fungi can affect mobility, transfer between biotic and

abiotic locations, and their significance in metal cycling in the environment: “The safe long-term storage of both existing and future nuclear wastes is of vital importance in protecting the environment” (Gadd, 2007).

Two ways fungi degrade mineral substrates are biomechanical and biochemical. Biochemical weathering can be direct or indirect. Direct biomechanical degradation of minerals can occur through penetration by fungal hyphae into decaying rocks by tunneling into intact mineral matter and can occur along crystal planes, cleavage, and cracks and grain boundaries, such as sandstone, calcitic, and dolomitic rocks. The fungal hyphae’s mechanical forces are derived from the osmotically generated turgor pressures inside the hyphae. The two main mechanisms of solubilization of rocks and minerals by fungi are acidolysis and complexolysis, which may be enhanced by metal accumulation in and/or around the fungal biomass. Many fungi can excrete metal-complexing metabolites (including carboxylic acids, amino acids, siderophores and phenolic compounds) are associated with complexolysis or ligand-promoted dissolution. Carboxylic acids derived from fungi with strong chelating properties aggressively attack mineral surfaces (Gadd, 2007). Gadd et al have explored the relationship between heavy metals (e.g., uranium, lead) and fungi (e.g., mycorrhizal). Below is a list of some of his case studies, each with a citation followed by a brief one-paragraph summary that could help the reader understand the relationship between fungi and heavy metals:

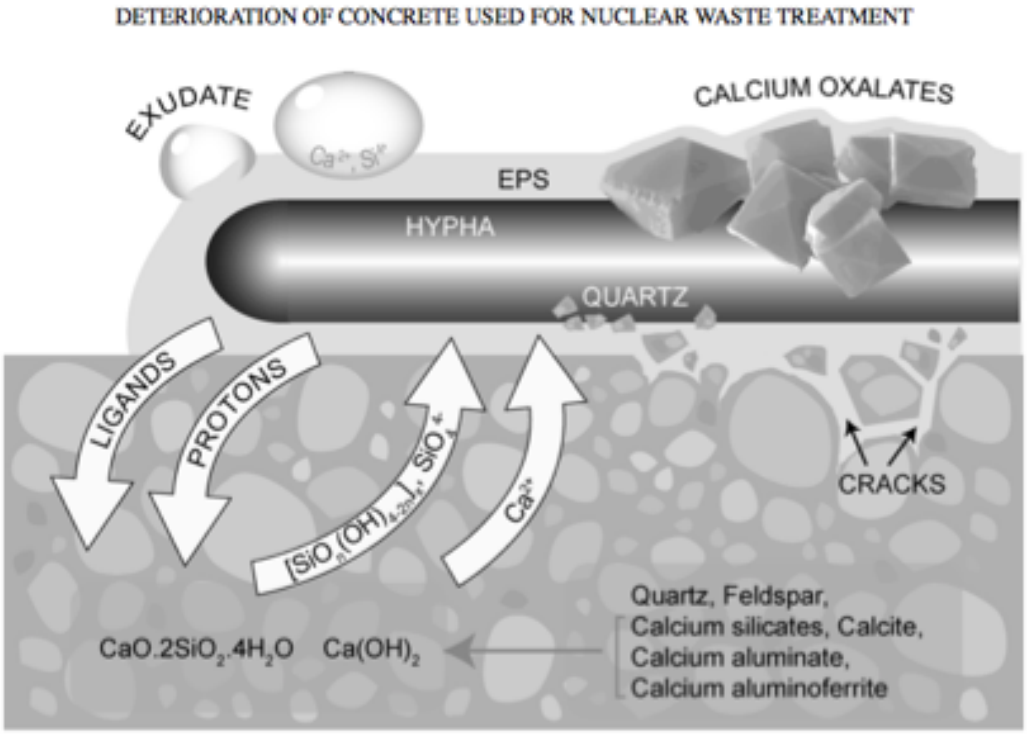
1. Fungal transformations of uranium oxides, 2007 (p. 1696-1710). In this study Gadd et al., revealed that fungi exhibit a high oxide tolerance, possess the ability to solubilize uranium trioxide (UO_3) and triuranium octaoxide (U_3O_8), and accumulate uranium within the mycelium. Uranium speciation, biomass showed in most fungi that

the uranyl ion was linked to phosphate ligands; however, in the ectomycorrhizal fungi a mixed phosphate/carboxylate correlation was observed. Abundant uranium precipitates associated with phosphorus were discovered in the mycelium encrusting the hyphae. Some fungi caused biomineralization of well-crystallized uranyl phosphate minerals of the meta-autunite group. They found all fungal cultures were highly tolerant to UO_3 and $U_3O_{8;}$ ectomycorrhizal *Rhizopogon* rubescens and all other fungi tested were completely tolerant. *Beauveria caledonica* increased mycelium density while others displayed no change. The highest uranium levels were observed in treatments with *B. caledonica* and *S. himantiodes* while the least were found in *P. simplicissimum* treatments. Mycorrhizal fungi accumulation of uranium was considered intermediate. In general, oxalate excretion by fungi increased over the first two-months and then decreased, with the exception of *S. himantiodes* which maintained high levels of oxalic acid excretion by the end of the fourth month. "Many free-living and symbiotic fungi are able to tolerate the toxicity of mobilized metals. Lichens are successful primary colonizers in extreme metalliferous environments and have been reported to grow directly on the secondary uranium minerals…" This study was the first experimental evidence that fungi transform uranium solids as well as they produce secondary mycogenic uranium minerals.

2. Fungal Deterioration of Barrier Concrete used in Nuclear Waste Disposal, 2007 (p. 643-653). Here, Gadd et al. investigated fungi's ability to biochemically breakdown barrier concrete used in nuclear waster disposal. They found fungi successfully dissolved cement with fungal biofilm and its associated microenvironment. Oxalate-excreting *Aspergillus niger* was observed forming plentiful calcium oxalate crystals on the concrete

and coated the fungi; see fig. 11: Deterioration of concrete used for nuclear waste treatment. Microorganisms deteriorate all different types of ceramic and building material including concrete and cement. Fungi can survive in and colonize concrete barriers under extremely radioactive contamination. For instance, in 1997-1998 extensive fungal growth was observed on the walls of the "Shelter" and other structures within Chernobly nuclear power plant building. High radiation pressure inside the No. 4 reactor caused genetically altered fungal strains of *Alternaria, Cladosporium*, and *Auzreobasidium*. Fungi are able to deteriorate concrete by chemical weathering of rocks and minerals, and the *Fusarium sp.* degradation of concrete proceeded more rapidly than bacterium-mediated *Acidithiobacillus sp.* degradation with complexolysis as the main mechanism of calcium mobilization. In this study scientists experimented with the following fungal species derived from toxic metal or radionuclide-polluted soils in the Ukraine: *Aspergillus niger, A. versicolor, Fennelia flavipes, Euro herbariorum, Paecilomyces lilacinus, Cladosporium cladosorioriodes, Alternaria alternata.* The fungal species listed above have been commonly reported to cause deterioration of building materials. Analysis of concrete matrix displayed quartz, feldspar, calcium silicates, calcite, calcium aluminate and aluminoferrite. Gadd's results showed colonization of fungi depends on concrete dimensions. Observed colonization of fungi avoided granite while some lichen hyphae avoided quartz. They concluded that these phenomena should be taken into account in exploiting concrete in nuclear waste disposal, in addition to other building contexts.

Figure 11: *Visual Aid Showing Concrete Deterioration*



Note. Simplified illustration of geochemical transformation of concrete by fungi. On surface of colonized concrete fungi formed a biofilm, which consisted of hyphae and extracellular polymeric substance (EPS), retaining moisture with exudate containing fungal metabolites. Mineral composites of quartz, feldspar, calcium aluminate and aluminoferrite with quartz and feldspar derived from added aggregates proved to be largely un-reactive. Fungi biochemically attack the concrete surface, excreting protons and ligands causing dissolution of concrete. First it reacts with cement paste components Ca $(OH)_2$ and cement paste calcium-silicate hydrate. The major element of cement mobilized calcium and silicon were leached from the concrete and accumulated within the biomass and exudates forming complexes with fungi metabolites and re-precipitated.

3. Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi, 2005 (p. 851-866). This study focused on the ability of ericoid mycorrhizal (ErM) and ectomycorrhizal (EcM) fungi to solubilize four toxic metals: cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn). Both the measurement of radical growth and biomass dry weight provided indications of metal tolerance. Metal accumulation in fungal biomass was measured using atomic absorption spectrophotometry. Solubilizing and metal tolerance varied widely between different fungal species and minerals as well as strains derived from sites with different degrees of metal pollution. Zinc phosphate proved the least toxic in addition to being the easiest to solubilize by the majority of tested fungal isolates. Solubiliziation of heavy metals was linked with pH of the medium and growth tolerance of fungi. It appeared that acidification of the medium was the main process of mineral dissolution for most of the mycorrhizal fungi used in this study. Scientists observed lethal effects for ectomycorrhizal isolates for greater than 60% of strains of Pb phosphate, carbonate, sulphide and tetraoxide. In contrast ErM isolates were able to grow on Pb-mineral-amended media. ErM cultures and 70-90% solubilized Cd and Cu phosphate and cuprite. Neither ErM and EcM produced a clear zone in Pb mineral-containing agar petridishes. "However, many fungi were able to accumulate mobilized Pb in their mycelia. Differences in toxic metal mineral tolerance, mineral solubilzation and metal uptake between populations isolated from metal-polluted and uncontaminated sites were related to the toxic metal which was the main pollutant in the contaminated environment. In general, the metal-tolerant fungi grew and solubilized toxic metal minerals better than non-tolerant isolates," (p. 851).

4. Role of fungi in the biogeochemical fate of depleted uranium (p. 375-377). Recent war campaigns in Iraq from 1991-2003 and Balkans 1995-1999 have caused dispersion of thermodynamically unstable depleted uranium (DU) metal into the environment. Even though DU is less radioactive than natural uranium, both have the same chemotoxicity and pose a hazard to human health. "Fungi are one of the most biogeochemically active components of the soil microbiota, particularly in the aerobic plant-root zone. [Gadd et al.] report[s] free-living and plant symbiotic mycorrhizal fungi has the ability to colonize DU surfaces and transform metallic DU into uranyl phosphate minerals" (p. 375). All fungi tested in this study displayed high DU tolerance and were able to colonize DU surfaces, forming moisture retaining mycelial biofilms. Fungi formed cord-like mycelial structures through aggregation of vertically aligned hyphae, which is commonly interpreted as a survival response to metal stress. Oxalic acid, a strong metal chelator, was produced by the DU-treated fungi and DU was observed to promote oxalate excretion. Gadd et al. suggests the role of oxalate in ligand promoted DU dissolution could be more significant than acidification; furthermore fungi *Beauveria caledonic, Hymenoscyphus ericae* and *Rhizopogon rubescens*, where exposed to DU showed increase accumulation of uranium along with increased amounts of excreted oxalate. "Metal immobilization can assist mineral dissolution processes and DU-exposed fungi demonstrated a remarkable ability to accumulate mobilized uranium in their biomass. Extensive uranium biomineralization occurred in all parts of the fungal colonies, from those regions adjacent to the DU coupons to even the remote marginal edges." (p.376). Fungi are particularly important in metal-rich and acidic soils. Gadd et al. were the first to show fungi can transform metallic uranium into metallic uranium into

meta-autunite minerals that have the ability of long-term uranium retention. Because of this and the fact that most terrestrial plant species are dependent on symbiotic mycorrhizal fungi, “this phenomenon could be relevant to the future development of various remediation and revegetation techniques for uranium-polluted soils” (p.375).

5. Geomycology: metals, actinides and biominerals, 2011 (p. 1-27). Fungi posses many properties that can effect changes in metal speciation, toxicity and mobility and mineral formation or mineral dissolution or deterioration. “Some fungal transformations have beneficial applications in environmental biotechnology, e.g., in metal and radionuclide leaching, recovery, detoxification and bioremediation, and in the production or deposition of biominerals or metallic elements with catalytic or other properties” (p. 1). Gadd et al. state, “[g]eomoycological roles of fungi have often been neglected in wider geomicrobiological contexts but they are of significant importance of in several key areas” (p. 19). Fungi are also being recognized for their importance within aquatic habitats specifically in sediments but their importance may be underestimated. Mutualistic relationships of fungi with phototrophic organisms, lichens and mycorrhizae are especially important as geoactive agents. Since fungi are found everywhere within the biosphere these processes emphasize the importance of goemycology as an interdisciplinary subject area within microbiology and mycology.

6. Lead Transformation to Pyromorphite by fungi, 2012 (p. 1-5). Lead (Pb) in all its chemical forms is a serious environmental pollutant. Metallic lead is an important structural and industrial material that is subject to weathering and also enters the soil

from hunting. Gadd et al. "… examined the influence of fungal [*Metarhizium anisopliae and Paecilomyces javanicus*] activity on lead metal and discovered that metallic lead can be transformed into choloropyromorphite, the most stable lead mineral that exists. This is of geochemical significance, not only regarding lead fate and cycling in the environment but also in relation to the phosphate cycle and links to microbial transformations of inorganic and organic phosphorous" (p.1). Gadd's report provides insight into mycogenic choloropyromorphite formation from metallic lead while highlighting its significance as a biotic component of lead biogeochemistry, along with additional consequences for microbe survival within lead contaminated environments and bioremedial treatments for Pb contaminated soils.

In the following sections I detail three mycoremediation case studies and one pilot project, and aim to provide you with examples of how mycoremediation experiments were carried out and the results.

First Example: Mycoremediation Pilot Project
Makah Nation's Tatoosh Island Neah Bay, Washington. 2009- present

The Makah Tribe places great cultural value on Tatoosh Island, and traditionally this island was a sacred site for burying the deceased. As mentioned earlier, mycoremediation is a comparatively inexpensive and effective new solution to the problem of oil contaminated soil and water, which impacts ecosystems health. Restoration methods first conducted by subcontractors working with the Department of Defense and the Makah Indians that removed polluted and contaminated soils from the island by helicopter were simple but cost excessive. They thus searched for a more functional, inexpensive, time conservative solution to their problems and considered mycorestoration

as the solution to contamination on Tatoosh Island. Before restoration could begin, however, more research and proof of concept using native species collected from Tatoosh Island were needed.

The Makah Environmental Restoration Team (MERT) has therefore been working for the last nine and a half years, exploring methods for restoring Tatoosh Island to conditions before Navy occupancy (RIDOLFI, 2009). Cerniglia et al. (2001) stated that abandoned military sites are usually contaminated by toxins ranging from petroleum, coal, and chemical residues. Contaminated soils on Tatoosh are extremely toxic. Tatoosh's landscape has steep cliffs that make accessing the island by boat or canoe extremely difficult. Appling clean up and in particular soil removal or replacement have proven extremely expensive and time consuming. Navigating around the rocks to a small beach by boat makes access difficult because of strong ocean currents. Thus helicopter transport is the preferred, although expensive, method of transportation. Removing a single cubic yard of diesel-contaminated soil off Tatoosh to the mainland a few miles distant costs approximately $1,000/ton. It is estimated that 300 tons of PCS (Petroleum Contaminated Soil) are present on Tatoosh Island. In need of a more cost effective solution, MERT believed solutions using variations of mycoremediation could be the key (RIDOLFI, 2009).

As described earlier, oyster mushrooms are white rot fungi, which are able to metabolize and digest PHCs "…by breaking apart the hydro carbon bond and releasing carbon dioxide and water" (Stamets, 2005). The prevailing hypothesis of how this occurs is that the hydrocarbons are demolecularized and re-assembled into fungal carbohydrates. Remediation efforts on Tatoosh Island currently focus on using the native fungal species

to reduce toxicities in the petroleum-contaminated soil. Several species of native mushrooms were collected for subsequent tissue culture isolation in October 2009. This approach is intrinsically less expensive because materials transported by helicopter would be drastically reduced. See Fig. 12 for photographs of Tatoosh Island; the native fungi we collected for lab analysis along with abundant salmonberry canes as possible substrate for inoculation while Tatoosh Island in ruins, Fig. 13: Native fungi *Marasmiellus* found on Tatoosh & Salmonberry canes.

After flying to Tatoosh Island with Ridolfi, Inc., the author and mycologist Paul Stamets to help collect native fungi and salmonberry canes, Fungi Perfecti successfully isolated pure cultures from native wild mushrooms. One species is particularly interesting because it was found growing on the salmonberry canes, the most abundant source of cellulose on the island. USDA scientists then showed its effectiveness in degrading PHCs in contaminated soils, and have documented this fungal species a *Marasmiellus candidus* senu lato. Fungi Perfecti proposed to use salmonberry canes as the cellulosic medium for inoculation. Since salmonberry canes are habitat for many different bird species participants must be careful not over harvest this resource and displace native bird species. Before use, salmonberry canes need to be rendered into a form so that mycelium will colonize. Sterilization by autoclaving is not practical for this site, but submerging canes in water for anaerobic fermentation or placing the salmon berry canes into .3% solution of hydrogen peroxide are cost effective methods of rendering this substrate useful for subsequent colonization by remediating mycelium.

In May 2012, Makah Tribe High School entered a contest through Samsung Electronics sponsored contest called *Solve for Tomorrow* and was awarded a $70,000

grant for the novelty of using mycotechnology. This multiyear project is on-going and will undoubtedly undergo revisions of methods depending upon results.

Figure 12: *Images of Tatoosh Island Neah Bay, Washington*

Note. From left to right, view of Tatoosh Island from helicopter, condemned buildings post navy occupany, imploded cement building, rusted bunker C oil barrel.

Figure 13: *Native fungi Marasmiellus found on Tatoosh & Salmonberry canes*
a.)

b.)

Note. a.) Native fungi *Marasmiellus* found on Tatoosh b.) Abundant salmonberry canes. Photos by Paul Stamets.

Second Example: Mycoremediation Case Study
Bioremediation of PAH-Contaminated Soil by Composting: A Case Study

Soil contaminated with PAH is of major concern and significance because of the toxicity, potential carcinogenicity and resistance to biodegradation. Cajthaml et al. (2002) *Bioremediation of PAH-Contaminated Soil by Composting: A Case Study*, a laboratory and field research project, was carried out to determine the degradability of 3 to 6-ring unsubstituted PAHs and other organopollutants within a composting system. Composting relies on the actions of microorganisms to degrade organic materials results in thermogenesis and the production of production of organic and inorganic compounds. Metabolically generated heat is concealed within the compost matrix and results in an increase in temperature.

The aim of this study was to determine the efficacy of composting the PAH-contaminated soil collected from a former tar production plant. Since scientists working on this case study used the same soil in another study where soil was treated by white-rot fungi, they were able to compare effectiveness of both composting and fungal treatments in PAH removal. Because the biodegradation of PAH can result in the production of metabolites more toxic than the original compounds, they also conducted an ecotoxicity test using luminescent bacteria and mustard seed.

During the composting soil mixing day, five random samples were taken for chemical analyses and toxicity testing. In conjunction with five samples from recently contaminated soil six samples were taken at different intervals within the compost pile. Samples were then air-dried prior to analysis of PAH and ecotoxicity. Out of the sixteen PAH samples described as important by the US EPA, eleven were analyzed throughout the duration of the experiment.

Study results revealed a 42-68% decrease in concentrations of phenanthrene, anthracene, flouranthene, and pyrene. The decrease in concentration of the higher-molar-mass PAH was lowered to 35-37%. Aside from the anthracene, no future decline in the concentration of other PAH was observed after maturation. However, in earlier experiments where the same soil was utilized for bioremedation involving two white-rot fungal species (*Irpex lacteus* & *Pleurotus ostreatus*) results showed that fluorine was decreased by (41 & 26%), anthracene by (29 & 19%), flouranthene (FLT) by (29 & 29%), pyrene (PYR) by (24 & 22%), and phenanthrene (PHE), displayed a 20% reduction by using only *Irpex lacteus*.

The fungi didn't degrade higher-molar mass PAH's. The comparison between bioremediation efficient of white-rot fungi and composting technology revealed composting was substantially more efficient in removing PAH, in reference to higher-molar mass compared to the fungal treatment.

Guerin (2002) compared two methods for removing PAH from aged soils of a former tar-contaminated site and found composting to be stronger technique than land-farming processes. Soil composting proved substantially more effective at removing higher-molar mass PAH. Over a seven-month treatment, removal by composting was 50% and by the farming technique the removal didn't surpass 5%. These results gave promise to Cajthaml et al. that their study would have similar reduction rates.

Comparative analysis of toxicity results obtained after composting and mycoremediation showed both treatments resulted in a decrease in toxicity of remediated soils as assayed by bioluminescence test on water elutriates.

Third Example: Mycoremediation Case Study
Use of fungal Technology in soil remediation: Water, air, and soil pollution, 2003

Sasek et al. (2003) conducted a case study using fungal technology in soil remediation. Many studies look at removing PAHs either with soil bacteria or with fungi but none to my knowledge combine these two methods. Moreover, most studies have been conducted in artificially PAH-contaminated soils. Sasek et al. focused on combining fungi and bacteria as bioremediation techniques in-situ. They utilized two white rot fungi: *Irpex lactetus* and *Pleurotus ostreatus;* a PAH-degrading bacterial strain of *Pseudomonas putida* was used as inoculum for bioremediation of petroleum hydrocarbon-contaminated soil from a manufactured gas-plant-landscape. After a 10-week duration of experiments, they found that, out of 12 different PAHs concentrations, four (phenanthrene, anthracene, flouranthene and pyrene) decreased up to 66%. The eco-toxicity of the soil after fungal-bioremediation did not reveal any detectable negative effects on the crustacian *Daphina magna*.

Sasek et al. (2003) used biological indicators to assess acute and chronic toxicity of PAH-contaminated soil before, during, and after remediation. PAHs degradation of soil toxicity was significant with reductions ranging from 35.2% to 92.3%. Ecotoxicity tests were performed on three different test organisms: luminescent bacteria, earthworms and mustard plant seeds (*Barassica alba*). A two-way analysis of variance (ANOVA) was utilized to evaluate effects on seed germination and earthworm survival. Soil was collected from a closed gas plant in Prague, Czech Republic, where total concentrations of PAHs were 609.8 mg kg^{-1}. The soil was also analyzed for cyanides and several heavy

metals (e.g., cadmium, cooper, mercury and lead). Fungal cultures were maintained on malt agar extracts. Finely ground, sieved wheat-straw was used as substrate for the inoculation of both fungi. The straw was moistened with distilled water and sterilized in an autoclave; fungi were grown on this substrate for two weeks prior to use for bioremediation in contaminated soil. In this experiment, Sasek et al. used seven different combinations with one as control. Fungi did not need to be reapplied but bacteria cultures were reapplied every 5 weeks.

Sasek et al. (2003) extracted and analyzed soil samples, and showed after 5 weeks that *I. lacteus* alone and co-cultured with *P. putida* were more effective in removing PHE (63%), FLT (15%), and PYR (36%). Before remediation techniques were applied, concentration of individual PAHs was respectively 100%. *P. ostreatus* and its co-cultures only reduced PAHs by 30%. Sasek et al. (2003) concluded that *I. lacteus* was more effective than *P. ostreatus* and bacterial cultures of *P. putida* in removing different kinds of PAHs. Applications of the two individual fungi with *P. putida* didn't improve degradation efficiency compared to fungi alone. Sasek et al remarked: "… this suggests that co-culture conditions with the respective fungi were not suitable for bacteria to take part in PAH degradation" (p.13). I suggest this was because the bacteria and fungi were outcompeting each other instead of metabolizing the PAHs.

Fourth Example: Mycoremediation Case Study
Mycoremediation: WSDOT 1996-1998 Case Study

In 1996, Paul Stamets in partnership with Battelle Pacific Northwest Marine Sciences Laboratories (MSL) conducted mycoremediation experiments using *Pleurotus ostreatus* to break down toxicities in diesel saturated soil and bunker C oil which was 30

years old (Stamets, 2006). As oil ages, it becomes more difficult to remove from soils. The oil and diesel contamination in these experiments was 20,000 parts per million (ppm) of total aromatic hydrocarbons (TAH), or about 2% of total mass. Concentrated contamination of this spill was comparable to the 1989 Exxon Valdez event where 11 million gallons of crude oil spilled into Prince William Sound. In 1998 the Washington Department of Ecology gave the Washington State Department of Transportation (WSDOT) the authority to conduct an experiment using mycoremediation. Researchers at the MSL in Sequim, Washington, teamed with Paul Stamets of Fungi Perfecti to test mycoremediation on this contaminated site (WSDOT, 1998, Stamets 2006).

WSDOT put four piles of diesel-contaminated soil – approximately 50 cubic yards each – onto individual 6mm black plastic polyethylene tarps. Piles were all of the same size, mounds sloping up to 4 feet high, 8 feet wide and 20 feet long. Into one of the four piles they mixed 3 cubic yards of cultured *Pleurotus ostreatus* sawdust spawn (Fig. 14), which is approximately equal to 20% percent of the pile's volume. A layering method was used, called parallel sheet spawning. This proved more effective as the mycelial fragments seek connection to one another. Two of the other piles were given bacterial and enzyme treatments while the fourth pile was left alone as an untreated control. The pile inoculated with mycelium had a shade cloth draped over it. The other piles were covered with black plastic tarps to avoid contact with rainfall. After a four-week period the piles were examined. The bacteria-treated and untreated piles of dirt were still black, smelled of diesel fuel and showed no signs of life, while the myceliated oil pile was lighter in color, lacked diesel fumes, and showed signs of myceliation. (Stamets 2006, WDOT 1998, Battelle 1999).

Upon examination, the scientists were astonished to see a flush of hundreds of *Pleurotus ostreatus (Pleurotus ostreatus)* mushrooms, some as large as twelve inches in diameter (Fig. 14). Stamets' text states this result can only be obtained if the substrate within the medium is nutritionally supportive for mushroom formation and development. Moreover, the soil that was once black had been transformed and become light brown and lacked the oil and diesel smells (Stamets 2006, WDOT 1998, Battelle 1999).

Another important aspect to this study is that the mycoremediated pile didn't require any additional maintenance; in contrast the WSDOT and sub contractor had to maintain the bioremediation- and enhanced-bacterial- applications in the other test piles. WSDOT applied 12 pounds of nitrogen fertilizers to the 50 cubic yards of contaminated soils under the assumption that the level of contamination was approximately 20,000 mg/kg TPH. Maintenance required monthly turning and additional fertilizers. WSDOT and PSCI Tank services applied enhanced bacterial treatments, which require biweekly and/or monthly applications of liquid fertilizer and bacterial inoculums along with biweekly or monthly rotations of the soil.

These experiments took place within a 16-week period from March to July 1998 (WDOT 1998, Battelle 1999, Stamets 2006). Scientists, however, speculated colder months during the experiment could have hindered the growth of the fungi. MSL cautions that variables to be considered are humidity, temperature of substrate, distribution of contamination within soil, and duration of treatment along with biological availability (Battelle, 1999).

After eight weeks the vascular plants (Fig. 14: Remediation of Aged Oil in Excavated Soil for WSDOT 1998) were growing and a community of insects and birds

were feeding off the mycoremediated pile (Stamets 2006, WDOT 1998, Battelle 1999). The fruitbodies of *Pleurotus ostreatus* sporulated at maturity, and thereafter began to decompose, primarily from bacteria and other fungi. (Stamets 2006, WDOT 1998). During decomposition many forms of life were attracted to this habitat, including insects, which in turn attracted birds. Birds feeding off insect larvae presumably left seeds, which could also have been windblown. The pile soon harbored a complex diversity of plants, bacteria, insect and fungal species (Stamets 2006, WDOT 1998).

Even though the insects and animals consumed the mushrooms, the researchers advised that humans, out of an abundance of caution, should not consume mushrooms from contaminated soils. Although there were no detectable petroleum residues in the mushrooms tested, analysis has shown an increase in heavy metals, which is cause for concern. The primary by-products from the mycelium were water and carbon dioxide because they are heterotrophic. The physical volume of the pile substantially shrunk in comparison to the other piles. Although the soil was not recommended for agriculture, nor were the mushrooms considered safe for human consumption, the biomass was approved for use in landscaping.

Battelle and WSDOT reported TPHs were reduced from 20,000 ppm to less than 200 ppm after a period of sixteen weeks, thus demonstrating mycoremediation effective in small-scale habitat recovery with the potential for habitat recovery on global scales. It is common knowledge in environmental studies that the more weathered and aged are petroleum-contaminated soils, the harder they are to remediate (WADOT 1998). Fig. 14 shows the mycoremediation process in chronological order from left to right; note the color and texture change over time.

Figure 14: *Remediation of Aged Oil in Excavated Soil for WSDOT 1998*

Note. From left to right, soils contaminated with 30-year-old Bunker C oil, inocluated pearl oyster mushroom sawdust spawn, mycoremedaiton pile control and bacteria enzyme treated piles, pearl oyster mushroom flush (fruiting), remediated pile. Notice other plants have begun to take hold. Photos by Susan Thomas.

Fifth Example: Mycoremediation

Compost-mediated Removal of Polycyclic Aromatic Hydrocarbons from Contaminated Soils, 2003

In this experiment Sasek et al. (2003) examined compost-assisted remediation for a manufactured-gas plant soil contaminated with polycyclic aromatic hydrocarbons within a thermally insulated composting chamber using mushroom compost that consisted of wheat straw, chicken manure, and gypsum.

Composting is a widely used practice to degrade solid waste materials like agricultural wastes, sewage sludge, and food waste. In more recent times, composting has been studied as a remediation technology for hazardous waste. Both laboratory and field scale studies have been carried out to determine the ability to degrade of PAHs along with other organopollutants within the composting system. PAHs are of especial concern at many sites, which includes wood-treatment facilities and manufactured-gas plants. Soil that is contaminated with PAHs is of major concern because its toxicity and potential of being carcinogenic in addition to being resistance to biodegradation. PAHs pose significant ecological risk therefore these compounds are listed on the EPA priority pollutants list which includes 129 different pollutants[2].

In this study initial degradation of individual PAHs was from 20-60%. At the end of the 54-day period they observed an *additional* reduction of 37-80% of PAH. Chemical analysis of the contaminated soil for PAHs, ecotoxicity tests on bioluminescent bacteria, earthworms, and plants seeds were measured both before and after decomposition. After decomposition scientists noticed inhabitants of bioluminescence declined, and no significant change in toxicity was observed for earthworm survival and seedling

[2] http://water.epa.gov/scitech/methods/cwa/pollutants.cfm

germination. Genotoxicity tests were performed on samples taken from different parts of the composted pile. Only after composting were decreases in genotoxicity observed in samples from the top of the composted pile.

Critiques of Mycoremediation Studies

The following section gives my personal critique of mycoremediation in reference to the case studies and pilot projects discussed in the above sections.

First example. *Makah Nation's Tatoosh Island Neah Bay, Washington 2009- present mycoremediation* pilot project. Unfortunately this project has another obstacle to overcome: upon conducting a test for measuring heavy metals Ridolfi found abundant amounts of lead, which cannot be degraded but could be captured and moved into another insoluble form by using fungi, thus preventing it from leaching back into the environment. This is a good example of why it is important to test for a variety of possible toxins on your site during the early phases of a remediation project. Since funds are usually tight and testing expensive, any new discoveries of toxins complicate remediation and increases expenses. Luckily other fungi have the ability to uptake heavy metals. For instance, Gadd et al. studied the influence of fungal *Metarhizium anisopliae* and *Paecilomyces javanicus* activity on lead metal and discovered that metallic lead can be transformed into choloropyromorphite, the most stable lead mineral that exists. I suggest that if no native fungi on the island have the ability to uptake heavy metals scientists need to consider non-native fungal species, like those listed in the above studies. That said, participants in the

Tatoosh mycoremediation project need to be cautious about introducing non-native species so not to disturbed the natural ecosystem of this small island.

For removal of petroleum in contaminated soils a *Marasmiellus candidus* senu lato could be utilized; USDA scientists documented its effectiveness in degrading PHCs in contaminated soils. However if this species cannot give the Tribe the results it wants then *Pleurotus ostreatus* or Irpex lacteus species are promising candidate fungi to be implemented.

Second example. Cajthaml et al. (2002) *Bioremediation of PAH-Contaminated Soil by Composting: A Case Study*. The goal of this study was to determine the efficiency of composting the degradation of PAH in contaminated soil collected from a former tar-production plant. Since scientists used the same soil in another study where soil was treated by white-rot fungi, they were able to compare the PAH-removal effectiveness by both composting and fungal treatment. The biodegradation of PAH may result in the production of metabolites more toxic than the original compounds; ecotoxicity tests were conducted using luminescent bacteria and mustard seed.

Out of the sixteen PAH's described to be important by the US EPA, eleven were analyzed throughout the duration of the experiment. They compared this study to control samples PAH analysis of composted material revealed a 42-68% decrease in the concentrations of phenanthrene , anthracene, flouranthene, and pyrene. The decrease in concentration of the higher-molar-mass PAH was to 35-37%. Aside from the anthracene no future decline in the concentration of other PAH was observed after maturation.

The fungi didn't degrade higher-molar mass PAH as readily as lower-molar mass chemicals. However, in their earlier experiments where the same soil was utilized for bioremediation, white-rot fungal species (*Irpex lacteus* and *Pleurotus ostreatus*) were able to remove fluorine (41 & 26%), anthracene (29 & 19%), flouranthene (FLT) (29 & 29%), pyrene (PYR) (24 & 22%), and phenanthrene (PHE) (20% by only *Irpex lacteus*). White-rot species were effective at removing PAHs in other past and future experiments conducted by Sasek, who was one of the leading scientists working on this study and concluded reduction in PAHs using fungi to be 35.2 -92.3%. I ask whether the mushrooms were utilized at a seasonally appropriate time and whether other environmental conditions like moisture and temperature were considered. How often was the compost pile turned? How substantial is it to have a reduction in high molar-mass when other PAHs reductions are apparent? Without this information, it is hard to determine how they can improve the study in the future.

Comparative analysis of toxicity results obtained after composting and mycoremediation showed both treatments resulted in a decrease in toxicity of remediated soils as assayed by bioluminescence test on water elutriates. They found using seed-germination tests that the actual soil toxicity was lowered only after mycoremediation.

Third example. Sasek (2003), *Use of fungal Technology in soil remediation: Water air and soil pollution,* utilized two white rot fungi *Irpex lactetus* and *Pleurotus ostreatus* with a PAH-degrading bacterial strain of *Pseudomonas putida* used as inoculum for bioremediation of petroleum hydrocarbon-contaminated soil from a manufactured gas-plant-landscape. After a 10-week duration of experiments, they found that, out of 12

different PAHs concentrations, 4 were decreased up to 66%. PAH degradation of soil toxicity was significant with reductions between 35.2% and 92.3%.

Fungi did not need to be reapplied when bacteria cultures were reapplied every 5 weeks. Extraction and analysis of soil samples showed after 5 weeks that *I. lacteus* alone and co-cultured with *P. putida* were more effective in removing PHE (63%), FLT (15%), and PYR (36%). *P. ostreatus* and its co-cultures only reduced PAHs by 30%. Sasek et al. (2003) concluded that *I. lacteus* was more effective than *P. ostreatus* and bacterial cultures of *P. putida* in removing different kinds of PAHs in this particular circumstance. Applications of the two individual fungi with *P. putida* didn't improve degradation efficiency compared to fungi by itself. Sasek et al remarked: "… this suggests that co-culture conditions with the respective fungi were not suitable for bacteria to take part in PAH degradation" (p.13).

I suggest fungi were not suitable to use with bacteria for PAH degradation because the bacteria and fungi were outcompeting each other instead of metabolizing the PAHs. Variables for fungi growth should be considered including humidity, temperature of substrate, distribution of contamination within soil, and duration of treatment along with biological availability. These variables could have had positive outcomes on degradation of PAHs. In future work I suggest they try remediation using fungi first and then apply bacteria subsequently. Other fungi I suggest utilizing for PAHs removal include *Bjerkandera adusta, Naematoloma (Hypholoma) frowardii, Serpula lacrymans,* and/or *Stropharia rugosoannulata.*

Fourth example. *Washington State Department of Transportation 1998 mycoremediation case study.* After mycoremediation of PAHs with *Pleurotus ostreatus* mushrooms – if analysis has shown there to be an increase in or presence of heavy metals, even though there may be no detectable petroleum residues – I suggest using a fungi species can mobilize heavy metals out of the soil. To determine which species to use depends largely on which heavy metals are present. Please refer to Figure xx: **Mushroom Species effects on Heavy Metals** for more information about which species are active against arsenic, cadmium, copper, lead, mercury, and radioactive cesium. This case study was fundamental to understanding that *Pleurotus ostreatus* mushroom mycelium has the ability to remove 30 year old aged PAHs out of contaminated soils. I would like to see another study similar to this but with the added factor of high concentrations of heavy metals, comparing and contrasting concentration levels both before and after mycoremediation. I recommend utilizing the natural symbioses of *Pleurotus ostreatus* and another fungal species that has ability to mobilize the heavy metal of concern.

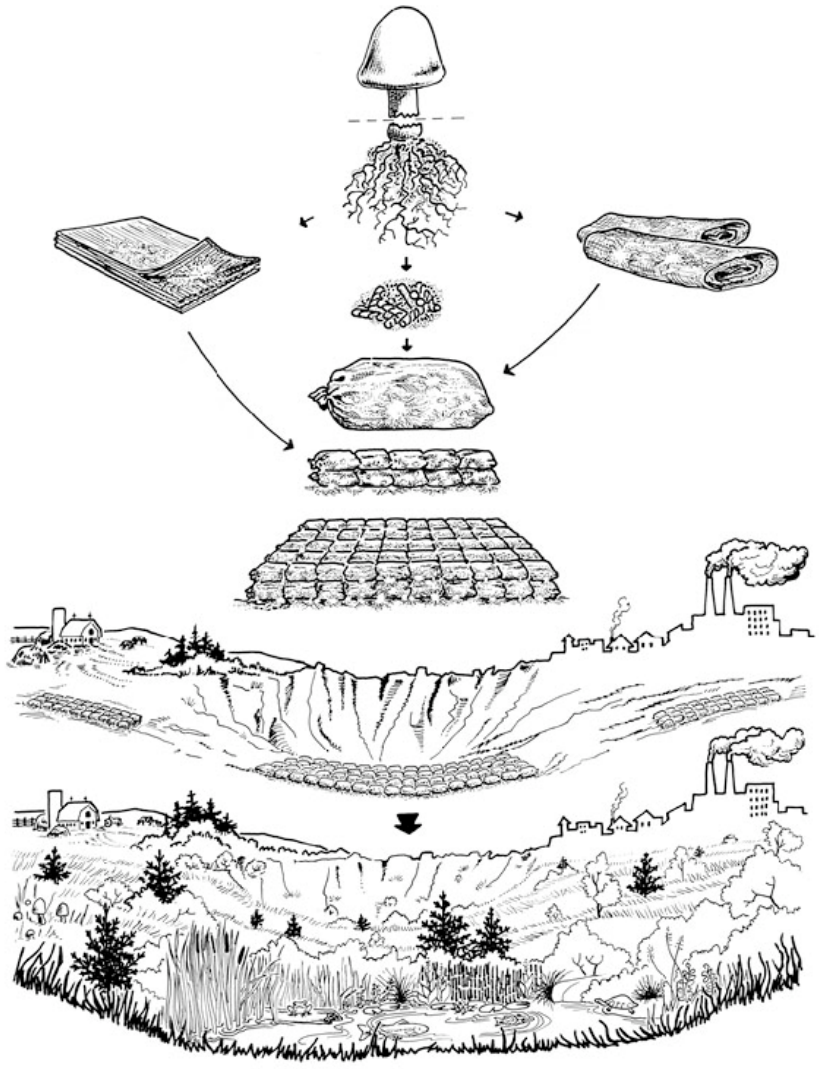
Fifth example. *Compost-mediated Removal of Polycyclic Aromatic Hydrocarbons from Contaminated Soils;* in this experiment Sasek et al. found degradation of individual PAHs was from 20-60%. At the end of the 54 day period they observed an *additional* reduction of 37-80% of PAH. Total PAHs reduction in this study was considerable. Both laboratory and field scale studies have been carried out to determine the ability to degrade of PAHs along with other organopollutants within the composting system. This study showed fungi's ability to reduce PAHs by a substantial amount. Future studies by Sasek should be referenced if one is interested in PAHs degradation by white-rot fungi. Had their testing

period been at least a year long, a degradation curve would have provided much more information about the duration of active decomposition beyond the confines of two month observations.

Pillar II: Mycofiltration

Mycofiltration (fig. 15: Simplified Illustration of Mycofiltration Installation process and fig. 16: Mycofiltration Symantec Installation) is a relatively new concept that could be installed into streams or ditches to filter harmful anthropogenic contaminants out of watersheds. The mycofiltration installation process is achieved by first identifying the contaminants at the site. Next, the remediating fungal species and delivery vehicle is chosen. For streams, ditches, and swales, burlap sacks filled with woodchips, and then inoculated with fungi, are preferred. Next, mycofiltration bags are strategically placed downstream. Four different options are illustrated in fig. 16 to help clarify installation can be found on page 118. Then mycofilter bags are secured by nails and stakes to prevent movement. Installation time is estimated at 45 minutes for 20-30 units. Cost of the mycofiltration installation is about $600 to $800 per site, which includes labor, equipment, and other materials not including inoculated burlap sacks donated by Fungi Perfecti (which would be $8/per mycofilter bag, with each weighing approximately 22 pounds).

Figure 15: *Simplified Illustration of Mycofiltration Installation process*



© Paul Stamets *from Mycelium Running,* 2005

Note. The above illustration shows a simplified version of the mycofiltration process capturing toxins from both industrial and residential pollution.

The Clean Water Act (CWA) of 1972 established regulations relating to discharge of pollutants into U.S waters, and also regulates quality standards for surface waters.

Under the CWA the EPA has the authority to set effluent limits on an industry-wide basis and has implemented pollution control programs as they relate to water quality, e.g., setting wastewater standards for industry. The EPA has also set water quality standards for all contaminants in surface waters. The CWA made it illegal to discharge pollutants from a point source into navigable waters unless a National Pollutant Discharge Elimination System (NPDES) permit is obtained. Point sources are discrete conveyances and include pipes or man-made ditches, individual homes that are connected to the municipal system, and septic systems. Industrial, municipal and other facilities must have a permit if their discharge goes directly into surface waters (Clean Water Act 1972). Alternative approaches for filtering water are bioretention cells (rain gardens), retention ponds, bioswales, water harvesting, sand filters, contour line infiltration trenches, small check dams, and straw bales. Bioswales are commonly found in urban areas to filter stormwater runoff.

First Example: Mycofiltration Pilot Project
The Evergreen State College Mycofiltration Pilot Project 2011- present

Tim Benedict, a Master of Environmental Studies graduate student, conducted bench test studies in The Evergreen State College (TESC) laboratory to see if a mycofiltration installation pilot project could be implemented in Snyder Creek on the Evergreen campus. Snyder Creek is commonly used to teach students how to conduct water quality testing. Benedict's paper examines fungi's ability to avert waterborne pollutants from entering the Puget Sound and its tributaries by using mycofiltration as a biological solution.

This study was conducted in collaboration with Fungi Perfecti, a company that is developing new concepts and methods of using fungi to degrade and sequester contaminates in our waterways. Benedict conducted controlled laboratory experiments with the Nisqually strain of *Pleurotus ostreatus* to assess the capability of using mycofiltration as a possible BMP. He found that filtering fecal coliform bacteria (FCB) contaminated water with nothing but woodchips reduced FCB concentration by 12%. However, when filtering through a mycofilter of sterilized alder wood chips inoculated with Nisqually strain of *Pleurotus ostreatus,* the fecal coliform count was reduced by 63%. While using non-sterilized alder wood chips inoculated with Nisqually strain of *Pleurotus ostreatus* the fecal coliform count was reduced by 87%. Benedict concluded that there was a 26% increase in effectiveness when using unsterilized woodchips colonized with *Pleurotus ostreatus* mycelium.

The second set of mycofiltration tests used two different substrates that were inoculated with same fungal species: straw and a sawdust/straw blend. In this portion of the experiment only sterilized mycofilters were utilized. Benedict found that FCB concentration levels fell below detectable levels and visually showed a significant reduction in fecal coliform bacteria. Benedict concluded that a "… mycelium network of *Pleurotus ostreatus* Nisqually strain efficiently filters and metabolically consumes fecal coliform bacteria. Mycofiltration has substantial promise for helping address fecal coliform contamination in the Puget Sound." He concluded that there are many advantages to mycofiltration: it is an effective, low cost, biological solution that requires minimal maintenance and is flexible for installation at a variety of sites. In addition, only decomposable byproducts are left by this application. The total costs of the lab supplies

were $300 for 200 mycofiltration tests (Benedict, 2011). He suggests further research to address flow-rates parameters and installation design, to both maximize filtration while allowing for appropriate oxygen levels. Evergreen's sustainability office showed interest in a mycofiltration installation at Synder Creek but felt it needed several more months of research. Benedict concluded that mycofiltration should be an ongoing project and should be applied as a BMP.

Second Example: Mycofiltration Pilot Project
Phase I (Proof-of-Concept) EPA Bench Study Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Stormwater, 2012- present

In February 2012, the EPA awarded Fungi Perfecti LLC a Phase I EPA SBIR $80,000 grant entitled "Comprehensive Assessment of Mycofiltration Biotechnology to remove Pathogens from Urban Stormwater". The research outlined in this grant will "… seek to identify which fungal species and cultivation methods can filter pathogens from stormwater while meeting the physical and temporal demands required for commercialization. These objectives will be accomplished through a university-industry collaboration that will mandate permeability and resiliency requirements for stormwater treatment. This research is anticipated to confirm that fungal mycelium can remove *E. coli* from flowing water, and that mycofilters can be developed to meet design requirements to treat municipal stormwater runoff." In addition to determining which species are optimal for mycofiltration in urban areas, Fungi Perfecti will experiment with both different combinations of species and substrate combinations. The following chart illustrates the different species and substrates for evaluation as mycofilters: 30 different combinations will be tested (Fig. 17: Fungal Species and Substrates Combinations). Note there are 17

mycofilters for each combination, 13 will be inoculated, while four will remain as controls in each batch.

Figure 17: *Fungal Species and Substrates Utilized in EPA Phase I*

Fungal Species	**Substrate used in Inoculations**
Pleurotus ostreatus (Pearl Oyster)	100% alder chips
Pleurotus ostreatus var. columbinus	50% alder chips, 50% alders sawdust
Ganoderma applanatum	50% alder chips, 50% rice straw
Ganoderma oregonense	50% alder chips, 25% alder sawdust, 25% rice straw
Trametes versicolor (Turkey Tail)	25% alder chips, 50% alder sawdust, 25% rice straw
Laetiporus sulphureus	

Note. Thirty different combinations of substrate and fungal species used in EPA grant Phase I. Table created by LaDena Stamets, reference Fungi Perfecti's EPA Grant 2012.

Fungi Perfect LLC has found three conditions that need to be effectively implemented in the field when utilizing mycofiltration; the installation must:

1. remain bactericidal for six-month duration of normal stormwater infrastructure maintenance cycles,
2. be capable of removing pathogens during high flow events, and
3. discharge treated stormwater with low pathogen concentrations.

To meet the three conditions above two key technical objectives need to be met. The first technical condition is to identify which species and filtration media combinations can maintain biological activity and appropriate permeability throughout the cycles of saturation, drying, heating, as well as freezing that will be encountered in mycofiltration installations. The second technical objective is to quantify the effects of mycofilters on bacteria. Then used as a model for pathogen filtration, the *E. coli* removal of the most viable fungal filter combinations is the first objective and will be evaluated at an average coliform runoff concentration (400 cfu/100mL) under an average size-adjusted Washington State six-month design storm hydraulic rate (2.2 L/min).

This research will also evaluate the presence of non-fecal coliform Klebsiella species bacteria, which is commonly found on wood. For example, the alder chips that will be utilized in this experiment either alone or in combination with other substrates will create the bulk of the mycofilters (EPA Grant 2012). Though testing for coliform is often considered by scientists an outdated, 20th century method to test if waters and shellfish harvest are safe, Fungi Perfecti will be able to eliminate "false positives" caused by this method of testing coliform bacteria as opposed to *fecal* coliform bacteria. If total coliforms are counted without discriminating between fecal and non-fecal coliforms, then the data could be skewed.

Mycofiltration is low-cost, low-impact and low-footprint technology. A recent analysis by Clary (2008) cited in the above grant proposal states "…analysis of stormwater treatment Best Management Practices (BMPs) has documented that coliform bacteria levels in treated effluent generally do not meet water quality standards," (EPA Grant no. SOL-NC-11-00012). This biological technology is competitive with other BMP

(Best Management Practice) currently utilized. For instance, the cost of sandfilters is $10,000-$20,000 with an annual maintenance cost of $3,000, while "mycofilters" only cost $8 each and maintenance cost are minimal. To determine how many mycofilters should be used in the field is largely a function of site characteristics. Throughout my research I saw that ten or more mycofilters are typically needed for the average site installation.

Once each mycofilter is fully colonized the three least colonized from each batch will be discarded. Of the remaining ten mycofilters six from each batch will be selected for a series of testing that includes: resilience, saturation, and permeability. These tests are aimed to simulate different climatic and environmental conditions. Resilience testing consists of cycles of saturation, drying, heating, and freezing as a way of evaluating their ability to maintain biological activity under field conditions. In saturation testing each mycofilter is submerged in water for thirty minute; mycofilters are then refrigerated at 4 ^{0}C for a two-day period. Mycofilters will then be kept at -20 ^{0}C for a 24-hour period, which will cause the mycofilters to dry. The next 24-hour period will induce a hot spell by raising the temperature up to 40 ^{0}C. For the next 24-hour period the mycofilters' temperature will be dropped by 10 ^{0}C. After cooling, the mycofilters will be submerged in water for twenty minutes. Lastly, mycofilters will be stored for 24-hours at 20 ^{0}C and 80% humidity. This will be the end of a 15-day testing period that concludes the resiliency testing process. From that point Fungi Perfecti will analyze one mycofilter from each batch for permeability and the other five will be evaluated for resiliency before field-testing. The strongest species substrate combination will be sent to Dr. Mark Beutel at Washington State University (WSU) for bacteria filtration efficacy testing. At this point

the controls will also endure the same test. Refer to figure 18 Mycofilter Resilience Testing Timeline for further explanation of this process:

Development of mycofiltration technology will result in a wide array of implications, commercial applications and benefits to society. Phase I, successful laboratory proof-of-concept data, will verify mycofiltration works. Phase II will advance development and field efficiency and support commercial development of mycofiltration as a low-cost, low-impact, and low-footprint application for removing pathogens from stormwater. The expected result of this research effort is a stormwater treatment system that will enhance the ability of municipalities both to improve quality of stormwater and to support a small innovative business. This biological technology will benefit society by providing cleaner water for commercial fishing and recreation. Moreover, this research could mobilize worldwide interest, engagement, and understanding in mycology, and provide innovation in solving complex anthropogenic contamination issues of non-point water pollution.

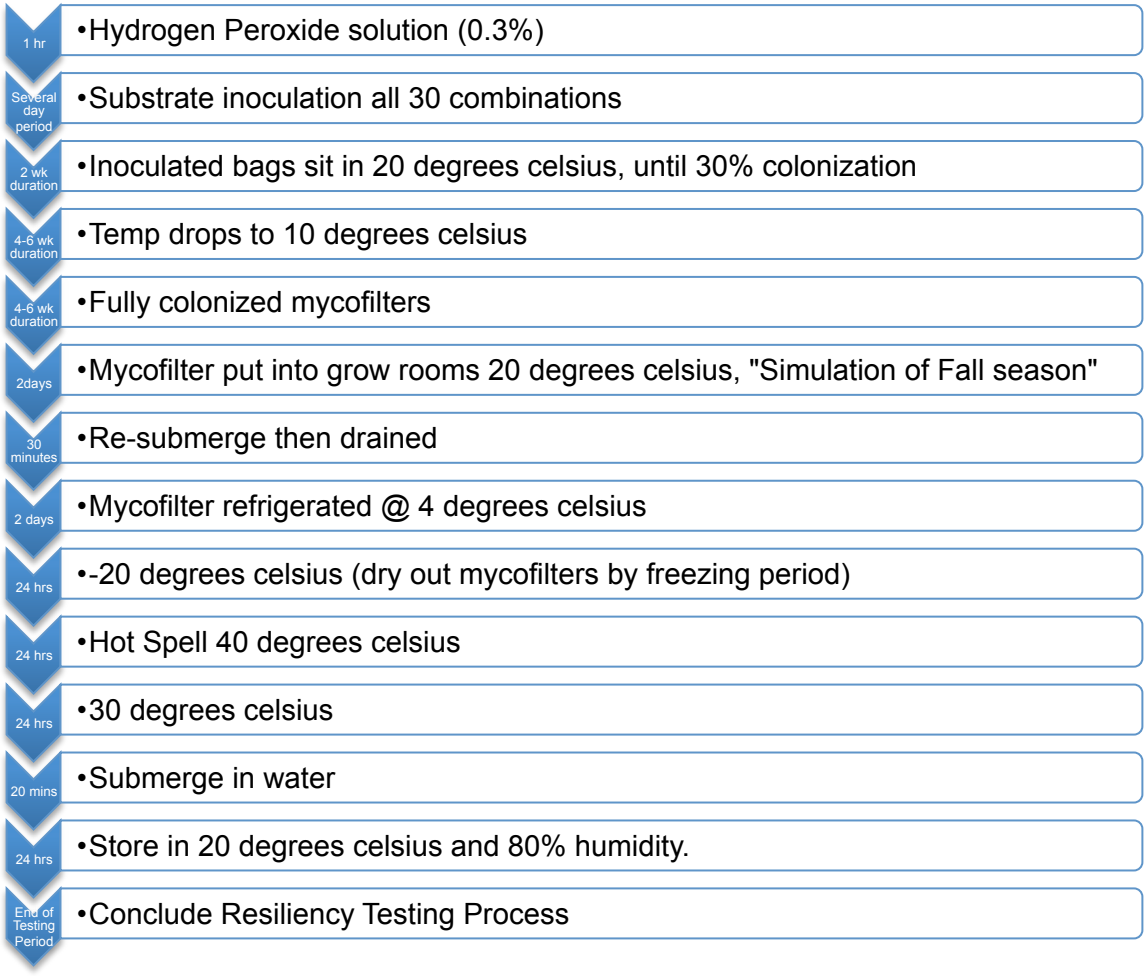
In Spring 2012 all companies awarded Phase I Small Business Innovation Research (SBIR) grants attended Phase I meeting in Washington D.C. Of 400 grant proposals, only 25 (6.25%) were selected. The SBIR grant program is aimed to enhance programs, open new markets and help existing businesses. In March 23, 2012 meetings with the EPA discussed the first phase of mycofiltration work. Many companies with promising technology will compete for the limited funding allocated in Phase II of the grant. If third-party investments totaling $100,000 are secured, EPA will include an additional $70,000 for successful proposals, which could potentially receive up to $300,000 for two years to develop the product further and bring it to market. Only 7 of 13

applicants will be funded for Phase II. The workshop focused on how to get the product to market, how to utilize EPA resources and meet deadlines as well as how get matching funds for Phase II. The following list shows potential target markets or consumers of vested interest for mycofilters installations:

- Life stock farms in violation
- Individual landowners of leaking septic tank violations
- "Buffers" or "riparian buffers" in National Forests where endangered or key-stone species live
- Native Tribal lands
- Commercial Fishing Industries
- State Agencies
- Watershed buffers
- Community garden buffers
- Individual landowners with adjacent neighbors who create pollution that travels onto their properties
- Housing developments with stormwater regulations or violations
- Landscape companies
- Horse boarders, horse pastures
- Agricultural runoff (cow, pig, etc.)
- Municipalities
- Conservation organizations
- Restoration organizations
- Land-use developers

The list above may help you the reader broaden your understanding of who may benefit from this biological technology in the future.

Figure 18: *Mycofilter Resilience Testing Timeline for EPA grant.*



Note. Mycofilter Resilience Testing Timeline for EPA grant. This timeline shows the sequential process Fungi Perfecti used during resilience testing in order to find the strongest combinations between the thirty. This timeline was created by LaDena Stamets in April 2012 to aid clarification of resiliency testing.

Third Example: Mycofiltration coupled with other ecological process Fisherville Eco-Machine Pilot Project 2006- Present

This project aimed to develop a complex ecological design that naturally evolves to maximize dynamic and diverse biological surface areas, to treat wastewater by converting contaminated water and sediment. In this project John Todd, who owns John

Todd Ecological Design, Inc., wanted to develop an "eco-machine" (fig 19: *Fisherville Mycocells Eco-machine*) that would rapidly biodegrade Bunker C oil. Bunker C oil is a toxic, tar-like, thick viscosity, residual material from the manufacture of petroleum products, and often used as a fuel source for ships and electrical power plants. When spilled into the environment it can create environmental damage, which persists for years in both sediments and soils.

Figure 19: *Fisherville Mycocells Eco-machine*

Note. Notice the transparent solar penetrated eco-machine cells with marsh plants and Algae. Photograph from John Todd and Eugene Bernat.

Todd, in conjunction with Eugene Bernat, designed and constructed a greenhouse based pilot system where a pair of eco-machines operated in parallel. To maximize both surface area and ecological elements, each system has four ecologically different cell types, which include:

1. Solar based cells which support algal turf communities, and *Physa gyrins* (pouch snails) inhabitants,
2. Higher plant based cells with marsh plants growing on rafts,
3. Open water fish dominate cells, and
4. Fungi dominated "trickle filter" cells designed to support rapid growth of fungi. mycelia (fig. 20: *Mycofilter Trough "Trickle-filter").*

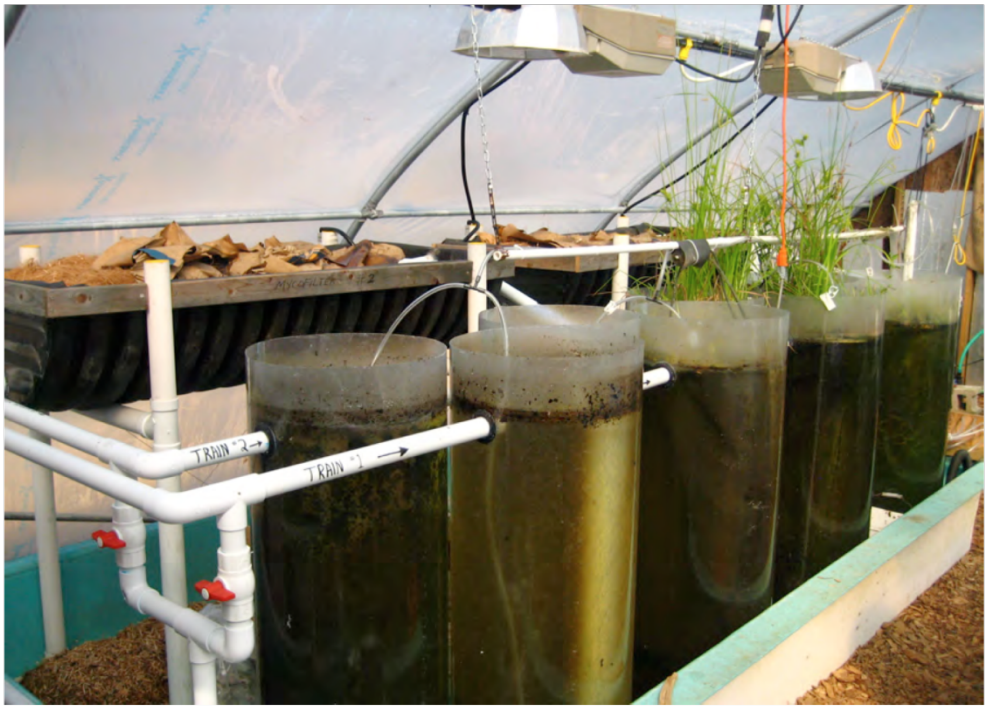
Figure 20: *Mycofilter Trough "Trickle-filter"*

Note. Fourth eco-machine cell inoculated with fungi. Photograph from John Todd and Eugene Bernat

Cell types 1-3 are contained inside clear transparent tanks to optimize solar penetration while the fourth cell type is covered to protect the fungi dominated system from light

penetration. These pilot systems are operated in a continuous recycle method. The system was inoculated with organisms from local salt marshes and ponds. In one of the Eco-machines a microbial chemostat was added to provide additional bacterial biomass on a consistent basis. A "train" is several eco-machines lined up to work in unison. See Figure 21: Eco-machine train.

Figure 21: *Eco-machine train*



Note. There are two Eco-machine Trains, with four eco-machines in each Train: (1) Solar based cells which support algal turf communities, (2) Higher plant based cells with marsh plants growing on rafts, (3) Open water fish dominate cells, (4) Fungi dominated "trickle filter" cells designed to support rapid growth of fungi mycelia. Photograph from John Todd and Eugene Bernat.

Ten pounds of sediment were extracted from the Fisherville Canal, divided equally into each of the two trains and added to the fungal cells. Over the next three months 573 gallons of water from the canal were divided between the two eco-machines. Each eco-machine's hydraulic capacity was approximately 200 gallons.

Before canal water and sediment were treated, scientists conducted chemical analysis. They found detectable measurements of chemical oxygen demand (COD), total suspended solids (TSS), alkalinity, ammonia, total kjehldahl nitrogen (TKN), and conductivity. The petroleum measurements included total organic carbon (TOC) in canal water and total petroleum hydrocarbon (TPH) found in both sediment and canal water. The first water tests in March 2007 revealed that TPH had dropped from 110,000 to non-detectable level and TSS decreased from 1,700 mg/l to a non-detectable level. Train #1 dropped from 1500 mg/l to 50 mg/l and train #2 dropped to 51 mg/l. Dilution alone had a 87% reduction of COD in both treatment train systems.

Approximately one month later, in April 2007, despite added contaminated canal water, COD dropped to 21 milligrams per liter (mg/l) in test one and 18 mg/l in test two. TPH water measured at 0.5 mg/l and 0.6 mg/l, thus the data for April showed a 92% decrease of TPH in the water. The sediment showed a significant reduction in volume. In Train #1 the sediment was reduced by 89% and in Train #2 by 57% reduction. Todd et al. observed that snails had begun to eat the Bunker C sediment attached to the side of the transparent tanks. A sample of the sediment revealed TPH of 66,000 mg/kg (a 40% reduction in Train #1), and 49,000 mg/kg (a 56% reduction in Train #2). “These numbers combined with the reduction in Bunker C sediment volume, indicate that the overall concept of the Fisherville Eco-Machine is valid and that the system is working” (as cited in Fisherville Eco-machine 2007 pp. 5).

Eco-Machine Design and Functions. How the infrastructure of the system is designed directly influences the ecological conditions of the eco-machine (fig. 19: Fisherville

Mycocells Eco-machine). Diversity in the physical characteristics of the eco-machine creates many different gradients of environmental factors such as light, oxygen, and turbulence and creates different niches for speciation that support a broad range of ecological communities. By having diverse ecologies, the eco-machine's functionality becomes both resilient and resistant to disturbance. This is significant because of the constant influx of highly polluted sediments and water into the eco-machines. The physical design was to maximize surface area to aid establishment of ecological communities, which are crucial to the function of the system. Transition zones are commonly referred to in the scientific community as "ecotones." Transitional zones within the eco-machines allowed the different ecological communities to interact. The pilot project design to include clear tanks, floating plant racks, screens, and mycofiltration troughs influenced the structure, composition, function and processes in the system that produced beneficial ecotones.

Floating racks suspend plants at the water-air interface. Growing vegetation created habitat niches for insects and for other organisms; the biomass of from the plants functions as a sink for nutrients derived from breakdown of the pollutants. The plants are self-organizing and self-repairing, their root matrices hosting communities of microbes that in turn either metabolize pollutants or externally degrade pollutants with enzymes. Microbial communities excrete simple compounds, which are then absorbed by the plant's root hairs and sequestered as plant biomass.

The mycofiltration troughs were housed within opaque materials to achieve a low-light environment and mimic a terrestrial environment, which creates a habitat that facilitate fungal growth. The substrate within the cells, coupled with different water

delivery systems, resulted in a “trickle-filter” corridor for water to pass through substrate colonized with diverse fungi and microorganisms, while maintaining high oxygen levels.

Participants of the pilot project allowed for a period of ‘ramp up’ during which the inoculated biology self-organized and established itself before being exposed to pollutants. After establishment, pollutants were gradually introduced and their concentration increased to give the ecological communities time to acclimate to the waste stream. Species were selected that tolerate the pollutants within the canal sediment and water. The project continues to adapt and evolve chemical conditions created by increased concentration of polluted canal water. At the time the 2007 report, the system was observed approaching, but has not yet reached, biological capacity. Additional inoculations of fungi will be necessary to introduce species of varying seasonality, and to increase both mineral and biological diversity within the system.

“As the biological capacity and full concentration of polluted water are reached, the ability to assess the design and operation variables influencing long term system functioning increases, providing key information for optimizing efficiency and minimizing costs of further efforts” (as cited in Fisherville Eco-machine Project, pp. 9). Initial positive results of this project demonstrated the need to continue the experiment and subsequent results confirm that this method offers a unique solution to a suite of chemically induced eco-challenges.

Ecological Development. Biological components of the system have self-organized into ecological communities that include food chains and symbiotic relationships. When interspecies interactions are considered it was found that species “x” only achieves high

population growth in the presence of species "y" even though species "y" may not break down pollutants alone. Managing the species that doesn't break down pollutants achieves better pollution reduction, higher adaptability, and increased resistance to disturbance (as cited in Fisherville Eco-machine Project).

Algae and snails. Biological cycles of algae blooms followed by pouch snail (*Physa gyrins*) population climax have been observed. At the same time that algae attached to the tank walls and continued to grow, the sediment contaminated with TPH suspended within the water and then became trapped where algal colonies experienced expositional growth covering most of the container surface area available. Scientists observed after several weeks that pouch snails began to scrub the tank walls by eating algae and sediment (as cited in Fisherville Eco-machine Project).

The EPA classify the pouch snail as a bioindicator generally associated with high nutrient levels. The snail population boomed initially with the available food source and then declined after the attached algae communities were consumed. Scientists hypothesize that this relationship is playing a major role in the reduction of TSS and TPH in the system, and suggest that this phenomenon opens a promising avenue of research options.

Fungi. Fungi species *Pleurotus ostreatus* and *Trametes versicolor*, which have the ability to degrade petroleum hydrocarbons, were inoculated within the substrate. As the mycelium mass expands it colonizes more substrate. The mycelium surface area was exposed to contaminated water and sediment inputs. Most white rot fungi secrete extracellular enzymes that can catalyze oxidation processes. Several mechanisms for enzyme secretion are utilized by *Pleurotus ostreatus* to oxidize PAH. Mycofilters

originally weighed five pounds each, and later, when mycofilters were excavated, sediment mass was only .549 lbs. from Train # 1 and 2.174 pounds from Train #2, equating to a reduction of respectively, 89% and 57%. Scientists also noticed that characteristics of the soil had changed in that it became more pliable and less sticky.

Plants. Plants have multiple cycling pathways that help influence microclimatic conditions, which vary by species. Plant roots are able to secrete enzymes that promote particular microbial diversity and activity depending on plant species and incumbent environmental conditions. Scientists suggest that "[h]aving these roots in an aqueous solution may have a similar enhancing effect on enzyme production as noted by Lenz and Holker for fungi. Matching plant adaptive strategies and chemical pathways to the challenge of degrading complex hydrocarbon chains poses great opportunities for further research. Plants are key to the photosynthetic base of the system and further diversify the system with habitats for terrestrial and flying insects, which play a yet-to-be understood-role in overall balance and health of system." (as cited in Fisherville Eco-machine Project pp. 11).

Water Quality Data. Raw canal water, before treatment, was tested on several water quality parameters with (TPAH) in the bunker C oil as a measurement of particular significance and interest. Alkalinity, ammonia, chemical oxygen demand (COD), total suspended solids (TSS), total kjehldahl nitrogen (TKN), conductance, as well as total phosphorous were measured. Refer to Figure XX: Water quality measurements from the Blackstone River Canal in Fisherville, MA (2006) below.

Figure 22: *Raw Canal Water Prior to Eco-machine Treatment*

Water Quality Measurement	Raw Canal Water Prior to Treatment
TPH (mg/L)	7.8
DO (mg/L)	9
Alkalinity (mg/L as CaCo)	14
Ammonia as Nitrogen (mg/L)	2.1
COD (mg/L)	1,500
TKN (mg/L)	17
TSS (mg/L)	1,700
Conductance (umohs/cm)	390
Total Phosphorous (mg/L)	15
Waste Strength	**100%**

Figure 23: *Water Quality Test 1: Train #1: Comparison of raw water from Blackstone River Canal to treated water from Fisherville remediation pilot eco-machine w/ reduction of pollutants (3-5-2007). Water Quality Test 2: (4-2-2007)*

Water quality measurement	Raw Canal Water	Lab test results for sample taken from Train #1, cell 4 on 3/5/2007	Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Percent Reduction of Pollutants
TPH (mg/L)	7.8	ND (RL: 0.5)	1.93	>74.15
DO (mg/L)	9	10		
Alkalinity (mg/L as CaCo)	14	11		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	0.52	>61.6
COD (mg/L)	1500	50	372	86.56
TKN (mg/L)	17	ND (RL:0.5)	4.22	>88.14
TSS (mg/L)	1700	ND (RL:5.0)	421.6	>98.81
Conductance (umohs/cm)	390	300		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	3.72	>86.56
Waste Strength	**100%**		**24.8%**	

Water	Lab	Lab test	Waste stream strength	Percent

quality measurement	test results for raw canal water	results for sample taken from Train #1, cell 4 on 3/5/2007	in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Reduction of Pollutants
TPH (mg/L)	7.8	0.5 (RL:0.20)	6.7	92.53
DO (mg/L)	9	9.8		
Alkalinity (mg/L as CaCo)	14	3.8		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	1.81	>88.89
COD (mg/L)	1500	21	1287	98.37
TKN (mg/L)	17	0.5	14.59	96.57
TSS (mg/L)	1700	ND (RL:5.0)	1458.6	>99.66
Conductance (umohs/cm)	390	460		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	12.87	>96.11
Waste Strength	**100%**		**85.8%**	

Figure 24: *Water Quality test 1: Train # 2: Comparison of raw water from Blackstone River Canal to treated water from Fisherville remediation pilot eco-machine w/ reduction of pollutants (3-5-2007). Water Quality test 2: (4-2-2007)*

a)

Water quality measurement	**Lab test results for raw canal water**	**Lab test results for sample taken from Train #2, cell 4 on 3/5/2007**	**Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity**	**Percent Reduction of Pollutants**
TPH (mg/L)	7.8	ND (RL: 0.5)	1.93	>74.15
DO (mg/L)	9	10		
Alkalinity (mg/L as CaCo)	14	15		
Ammonia as Nitrogen (mg/L)	2.1	0.3	0.52	42.4
COD (mg/L)	1500	51	372	86.3
TKN (mg/L)	17	1.1	4.22	73.9
TSS (mg/L)	1700	ND (RL:5.0)	421.6	>98.8
Conductance (umohs/cm)	390	300		
Total Phosphorous (mg/L)	15	0.7	3.72	81.2
Waste Strength	**100%**		**24.8%**	

b)

Water quality measurement	Lab test results for raw canal water	Lab test results for sample taken from Train #2, cell 4 on 3/5/2007	Waste stream strength in eco-machine at sampling time as a function of waste water percentage of whole system hydraulic capacity	Percent Reduction of Pollutants
TPH (mg/L)	7.8	0.6 (RL:0.20)	6.7	91.03
DO (mg/L)	9	9.8		
Alkalinity (mg/L as CaCo)	14	3.8		
Ammonia as Nitrogen (mg/L)	2.1	ND (RL: 0.2)	1.8	>88.9
COD (mg/L)	1500	18	1287	98.6
TKN (mg/L)	17	ND (RL:0.5)	14.59	>96.6
TSS (mg/L)	1700	ND (RL:5.0)	1458.6	>99.7
Conductance (umohs/cm)	390	470		
Total Phosphorous (mg/L)	15	ND (RL: 0.50)	12.87	>96.1
Waste Strength	**100%**		**85.8%**	

Sediment Data. Sediment from the Blackstone River Canal was tested for both TPH and TOC. TPH in the sediment was 110,000 mg/kg while the TOC was 130,000 mg/kg. Of the five pounds initial sediment added to train #1 and train #2, scientists observed mass reduction. At the end of test period 2, the sediment in Train #2 weighed 89.02% of the original weight, leaving only 0.549 lbs. sediment. The texture and viscosity of the sediment displayed considerable change from the original sediments. Treated sediment was softer, less sticky, and lost some of the metallic luster characteristic of petroleum. The small quantity of sediment that settled out in the aquatic cells was tested for TPH in both trains #1 and #2. A mixed sampling from all aquatic cells revealed a TPH concentration of

60,000 mg/kg in train #1 and 49,000 mg/kg in train #2. When these data were compared to the raw canal sediments, the reduction was approximately 45.5% and 56%.

Fisherville Eco-machine Conclusion & Further Recommendations. Fisherville Eco-machine Pilot project contributed measureable empirical data towards establishing the efficacy of the living system approach to treatment of oil contaminated canal sediment and poor river water quality. This project also revealed insight into appropriate optimal design of eco-machines to treat contaminants in the Blackstone River Basin.

The eco-machine project demonstrated significant reductions in both sediment mass and pollutant concentrations within sediment and water. Significant biomass was produced in an environment poor in nutrients excluding inputs of polluted water and sediment, and validated the research hypothesis that a complex ecology designed in conjunction with natural principles of evolution to maximize dynamic biological surface area and including waste water/sediment contact is capable of utilizing and converting contaminated water and sediment to an energy source readily available for biomass production. During the duration of the study scientists observed life colonization in all areas of the eco-machine trains. They noted that the thick algal mats that had covered almost all of the aquatic tank's walls were entirely consumed and metabolized by snails. Plant shoots grew to three-feet tall while their plant roots masses were thick and reached to the bottom of the five-foot enclosures. Even though the scientists didn't achieve completed colonization of the mushroom substrate they did observe significant patches of *Trametes versicolor* beginning to take hold in the system. Suggested fungi could have

played a more significant role in pollutant degradation through optimizing the mycofilters if they were well established before installation.

Future investigation of how pollutants were converted could explain exactly what paths the contamination was taking but scientists acknowledge that carbon isotope tracing testing is expensive and somewhat unnecessary since the process can be observed directly.

Scientists measured an 89% reduction of sediment with a TPH concentrations of 110,000 mg/kg in Train #1, and a 56% reduction in Train #2. Sediment characteristics changed, indicating some degraded of TPH but unfortunately they didn't have enough funding at the time of this study to conduct a chemical analysis of the remaining sediment. In addition, sediment that settled out of the water column had significantly lower concentrations of TPH, a reduction of 45.5% and 55.5 % compared to the sediment that settled in Fisherville and coated the Blackstone River Canal channel.

Water quality tests showed even greater removal of pollutants. TPH reductions were measured at less than 74.2% in both Trains #1 and #2 for test period one. For test period two approximately 92.5% and 91% reductions of TPH while COD and TSS were reduced by 99%. These large reductions in targeted contaminants of interest demonstrated the efficacy of Eco-machine technology to treat polluted waterways. Eco-machines' team of scientists attributes much of these reductions in toxins to the combination of algae-snail cycles, fungi, and the interaction of the dynamic root surfaces of the higher plants and their associated microbial communities with the water.

This study provided information on how to implement either direct or full-scale systems. The project showed how the technology using native organisms from all five kingdoms of life works, removing contaminants from both water and sediment. The

insights gained from this study will be utilized to optimize the technology and quantify appropriate scale and treatment rate for a full-scale system.

This technology is adaptable and can be scaled up or down to adapt to changing conditions or needs with minimal expenditure. It is also applicable to large-scale watershed restoration because of its ability to be moved. The study authors envision that the Eco-machine hybrid can be broken down to small functional units, which can be located up and down the stream of the watershed in situ to deliver treatment where it is most needed. Units can be linked to handle high loads where pollution is abundant, then split into independent, smaller units to clean less polluted areas in unison to neutralize pollution hot spots. This approach would keep costs low, while also making treatment adaptable to conditions in the field. Furthermore, once the restored area reaches desired reduction in contaminates, these units can be transferred to other restoration efforts and continue to clean polluted waters beyond the scope of a single project.

Fourth Example: Mycofiltration Case Study
Mason County Mycofiltration Projects, 2008 -2011

In 2011, Mason County had several ongoing mycofiltration installations (see fig. 25: *Mycofiltration Sites locations for Hood Canal, Mason County*) but all except one were discontinued due to lack of funding. At the time federal funding had been allocated to Squaxin Tribe's marine biologist John Konovsky for a mycorestoration project in a polluted waterway in Allyn, Washington. The Allyn site has become a high priority for Mason County and lessons learned from the prior sites will be applied to this and new mycofiltration installations (Book et al., 2009).

Figure 25: *Mycofiltration Site Locations for Mason County Installations*

Mycofiltration Sites Locations for Mason County	
Annas Bay	
	Hwy 101 near Finch Creek, Hoodsport
	Two sites on State Route 106 near Skokomish River on Warren Drive 1/2 mile apart
	Main Street
Oakland Bay	
	251 Sunset Road
	State Route 3
	520 Eckler Road (SR3 ROW) at creek
Belfair	
	Roessel Road
	Hwy 3 Log Plaza
	Hwy 3 HC Auto Sales
	Old Belfair Hwy
Other sites	Chapman Cove
	18280 SR3, Allyn
	221 E. 4th Street, Union

Note. Table created by LaDena Stamets reference Quality Assurance Project Plan for Mason County Mycoremediation Investigation.

The Squaxin Tribe was interested in this biological solution for capturing and removing bacteria from running water. Mycelia acts as a biological filter capturing fecal bacteria from human and livestock waste in stormwater runoff, which is one of the most widespread contributors of water pollution. It was hoped that mycofiltration could be utilized to protect the rich shellfish heritage of the Puget Sound, which is historically,

culturally and economically important to Salish Tribal communities. Runoff pollution into Puget Sound from upland transportation each winter is a common cause of closing shellfish harvest. Shellfish growers fear that this yearly cycle of pollution will negatively impact economic development; as Konovsky states, "We need innovative and cost-effective methods to solve the problem." Squaxin's benchmark for a cleanup Puget Sound is whether the Natives can eat its shellfish and harvest healthy populations of salmon. If so, then mycofiltration can be a valuable tool to aid the clean up of Puget Sound (Squaxin mushroom water quality solution, 2011).

Due to hydrological characteristics of the design, influent water traveling pooled behind mycofiltration bags and then slowly filtered through the bags as it flows down to lower elevations. Mycelium filters toxins out of the water by capturing them in its cellular net and then digesting them as a nutrient source (Book et al., 2009).

In February 2010 a mycofiltration meeting of State Public Works, Conservation District officials, Squaxin's Fish Biologists, and Fungi Perfecti included discussions about the limitations and concerns with the data collected. One issue with the burlap bags was that the mycelium, at times, dies off; another was that extra debris and sediment prevents water from flowing. It is important that the sacks be lifted out of waterways without falling apart. Heavy water flows have caused bags to blow out. Options to ameliorate these issues include hemp sacks, thicker threading on burlap sacks and use of the plastic netting commonly used in the shellfish industry. Introducing plastic netting into the environment is not favored because the plastic might fragment into smaller pieces that could later consumed by riparian creatures and fish. Since mycofiltration is a biological solution, it is important that personnel pay attention to natural life cycles and climatic

conditions. Data show that while mushrooms thrive between the months of September and December, the mycelium grows well from March through November, so the incubation of substrate inoculated with mycelium can be without climatic controls 75% of the time in the PNW (Book et al., 2009). This is important to note because mycofiltration installations need to take place during seasonally appropriate times to maximize effectiveness.

From the meeting, Stamets' team focused on two issues concerning how the mycoremediation was being carried out: 1) the way the bags were being stored before installation hindered their effectiveness, and 2) bags were not being replaced within the prescribed time period. Study participants agreed that these several pilot studies needed to be better documented, and that more controls needed to be in place. Several fungal species were included in some bags, and data suggested that the strongest strain was *Pleurotus ostreatus* (the Nisqually Oyster strain). Some results showed a reduction in fecal coliform bacteria (FCB) while others showed an increase in fecal matter. Study participants speculated that animals such as mice were leaving fecal matter on bags. Other suggestions were to have a few control bags with just sterilized wood chips to provide an *in-situ* control, against which toxicity data could be compared. Thus methods used in this case study need to be further researched and developed before being designated as a Best Management Practice (BMP) by the EPA (Book et al., 2009).

Fifth Example: Mycofiltration Pilot Project
Field Demonstration of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington 2009

Battelle Pacific Northwest National Laboratory in partnership with the U.S. EPA and DOE integrated fungi into bioretention cells in efforts to reduce FCB contamination in the Dungeness Watershed. Mycofiltration reduced FCB by 90-97% when using native

vegetation and natural microbial assemblages, while the control bioretention cell without fungi reduced FCB by only 66-73%. Thus, it can be seen that fungi significantly increased the effectiveness of the bioretention cell by 24% (Thomas et al. 2009).

The Dungeness Watershed is located within the Olympic Peninsula of the northern part of the Puget Sound. Rivers originate from the Olympic Mountains and flow 32 miles downstream through wilderness, forested, agricultural and residential landscapes into Dungeness Bay. The 200 square mile watershed is habitat for over 200 fish and wildlife species and is an important waypoint for migratory waterfowl.

Dungeness Bay is located within the Dungeness National Wildlife Refuge and provides refuge and nursery grounds for native birds, fish and shellfish species. For over 20 years, a collaborative effort between local and regional institutions as well as other collaborative partnerships worked to maintain ecosystem function. Ecosystem functionality is dependent upon the interactions between organisms and the physical environment, such as nutrient cycling, soil development, water budgeting, and flammability in the Dungeness Watershed. In recent years, anthropogenic impacts impaired natural function of both river and bay causing multiple health problems, including the listing of salmonid species under the Endangered Species Act and, since 2000, closure of Dungeness Bay shellfish harvesting due to high levels of fecal coliform bacteria. Although some improvements have been made, failing septic systems, impaired in-stream flows, pollutant inputs caused by stormwater runoff, and flood plain development continue to threaten habitat health. This study took place on a residential property in an agricultural setting in the lower Dungeness Watershed. At one point, the site had been utilized as an irrigation overflow pond. This site was adjacent to pasture land

and tidal wetland that is contiguous to the Strait of Juan de Fuca.

The study *Field Demonstration of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington* is technically, a mycofiltration study, not a mycoremediation study, as mycofiltration captures pathogens flowing with water whereas mycoremediation which degrades arrested pollutants in situ. . Thomas et al. focused on mycofiltration used in conjunction with bioretention cells as a possible Best Management Practice to remove fecal coliform bacteria. They used a bioretention cell (rain garden) as a control and a bioretention cell coupled with fungal mycelium and mycorrhizae as the treatment. Their design protocol was to see if adding the bioretention cell infused with fungal species *Pleurotus ostreatus* and mycorrhizae increased the removal of fecal coliform bacteria. The study site received runoff from an irrigation ditch. Scientists spiked the experiment with fecal coliform, which allowed a comparison of treatment with control. This study is a part of the larger body of research conducted under the funding of the EPA Targeted Watershed Initiative within the Dungeness Watershed and Bay to encourage community based solutions to protect and restore clean surface water.

The mycofiltration application consisted of a layer of oyster mushroom (*Pleurotus ostreatus*) mycelium to inoculate alder (*Alnus rubra*) chips mulch and mycorrhizal fungi applied to plants. Refer to Figure 26: *Native plants were used in the biofiltration cells,* which includes a list of species of trees, shrubs, emergent, and herbaceous plants used in this study.

Figure 26: *Native Plants used in Biofiltration Cells*

Native plants used in biofiltration Cells, Dungeness Watershed

Plant Type	Genus/Species	Common Name
Trees	*Malus fusca*	Pacific crab apple
	Salix lucida	Shining willow
	Crataegus douglasii	Black hawthron
Shrub	*Cornus sericea*	red-osier dogwood
	Lonicera involucrata	twinberry honeysuckle
	Myrica gale	Sweetgale
	Physocarpus capitatus	Pacific ninebark
	Oemleria cerasiformis	Indian plum
	Symphoricarpos albus	common snowberry
	Ribes lacustre	black swamp gooesberry
	R. sanguineum	red-flowering currant
	Crataegus douglasii	black hawthron
	Spiraea densiflora	rosy spiraea
	S. betulifolia	white sporaea
Emergent	*Carex lyngbyei*	Lyngbye's sedge
	C. mertensii	Mertens' sedge
	C. obnupta	slough sedge
	C. pachystachya	chamisso sedge
	C. pansa	sanddune sedge
	C. sitchensis	sitka sedge
	C. spectabilis	showy sedge
	Eleocharis palustris	common spikerush
	Juncus effusus	common rush
	J. tenuis	proverty rush
	Scirpus microcarpus	panicled bulrush
Herbaceous	*Aster chilensis*	Pacific aster
	Iris tenax	thoughleaf iris
	Sisyrinchium angustifolium	narrowleaf blue-eyed grass
	Fragria chiloensis	beach strawberry
	Potentilla fruitcosa	shrubby cinquefoil

Note. Table created by LaDena Stamets. Reference: *Field Demonstration of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed*, Washington 2009.

Study authors found the bioretention cell alone decreased fecal coliform count-forming-units (CFU's) by 66%, but the bioretention cell infused with mycorrhizae reduced CFU by a additional 29%, for a total 90% reduction. The bioretention cell outflow revealed an initial spike of 376 CFU/100 ml at one hour, and then consistently dropped over time. In contrast the mycoremediation outflow showed concentrations no greater than 10 CFU/100 ml and remained relatively constant through the duration of the experiment with an average mean of 5 CFU/100 ml. Thomas et al. (2009) concluded: "While the bioretention cell itself performed well at reducing fecal coliform bacteria, the mycoremediation (sic) treatment provided a greater reduction of bacteria. This was particularly evident during the spike experiment where a higher concentration of bacteria and nutrients were introduced into the cells." Thomas et al. outlined the benefits of the mycofiltration treatment application to a bioretention cell or other type of site (e.g., stream bank, riparian buffer) which include:

- A technologically based natural systems,
- Only native fungal species used; can locally source all materials (plants and fungi),
- Minimal handling and low maintenance,
- Visible improvement to a site,
- Non toxic byproducts; no secondary waste streams produced,
- Local water quality protected,
- Mobile and flexible; no structures, no minimum batch size,
- Economical,
- Effective at reducing fecal coliform and nutrients when properly designed, and

- Applicable to a variety of other contaminants (e.g., PAHs, PCBs, metals).

For more information about this study I encourage you to read this field demonstration study.

Critiques of Mycofiltration Studies

The following section aims to give my personal critique of mycofiltration reference to case studies and pilot projects examples discussed in detail in the above sections.

First example. *The Evergreen State College Mycofiltration Pilot Project.* I found this study helpful in highlighting that mycofiltration was more effective when woodchips were not sterilized. I suggest this was because other naturally occurring microbial organisms were established and either aided in remediation or became food source for *Pleurotus ostreatus* mushrooms. This is helpful information for individual land-owners who might not have the ability or funding to sterilize would chips before inoculation.

I recommend that The Evergreen State College implement this mycotechnology at Snyder Creek as a field study – especially since students are already conducting water quality tests regularly in Snyder Creek. Students would take samples above and below mycofiltration installation for multiple quarters, then compile data into a useable form.

Second example. *Phase I (Proof-of-Concept) EPA Bench Study Comprehensive assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Stormwater.* The studies being carried out by Fungi Perfecti in collaboration with WSU

and EPA are fundamental to understanding which species and substrates will provide the most effective product for cleaning up fecal coliform bacteria in both urban and agricultural settings. The value of this research effort is a potential stormwater treatment system that would both enhance the ability of municipalities to improve quality of stormwater and supporting a small innovative business. A recent analysis of stormwater treatment BMPs has documented that coliform bacteria levels in treated effluent generally do not meet water quality standards. This research will also evaluate the use of non-fecal Klebsiella species bacteria, which are commonly found on wood, but are not harmful to humans. For example, alder chips will be utilized in this experiment, either alone or in combination with other substrates to create the bulk of mycofilters. Although testing for total coliforms, without distinguishing between fecal versus non-fecal coliform, is often considered by scientists an outdated, 20th century method to test if waters and shellfish harvest are safe, Fungi Perfecti approach was able to eliminate "false positives" caused by this antiquated testing method. Measurements of total coliforms, without distinguishing fecal and non-fecal coliforms used in mycofilter effluent skewed efficacy data. False positives have been a major obstacle in past mycofiltration water quality assessment with local state governments, particularly Washington State DOE, which has historically depended and currently depend upon on a flawed testing methodology. WSDOE needs to conform to the practices recommended by the EPA, to which the majority of states have also complied by updating their testing protocols.

Mycofiltration is low-cost, low-impact and low-footprint technology. Moreover, this research could mobilize worldwide interest, engagement, and understanding in

mycology, and provide innovation in solving complex anthropogenic contamination issues of non-point water pollution.

I suggest participants seek private investors for Phase II of the EPA grant and that a Mycorestoration Certification Training Program be established to establish standard protocols, hands-on training, and documentation. If private investors – especially stakeholders – shared financial responsibility for cleanup, then the EPA will match that funding for implementation of mycofiltration for urban stormwater. The Puget Sound area would be an optimal place to carry out this study because it has a massive human population living on thousands of miles of waterfront properties, especially considering that about 150,000 pounds of untreated toxins go into the Puget Sound each day.

Third example. *Fisherville Eco-Machine Pilot Project* is of considerable importance to bioremediation technologies that are coupled with mycofiltration's ability to ameliorate toxins in contaminated waterways. A complex ecology designed in conjunction with natural principles of evolution to maximize dynamic biological surface area and including waste water/sediment contact can utilize and convert contaminated water and sediment to an energy source readily available for biomass production. The Eco-machine Pilot project contributed empirical data demonstrating the efficacy of the living system approach to treatment of oil-contaminated canal sediment and poor river water quality.

Even though the scientists didn't achieve complete colonization of the mushroom substrate they did observe significant patches of *Trametes versicolor* beginning to take a strong hold in the system. I suggest in their next experiments to let fungi within the "filter trickle" feeder establish itself before introducing contaminated canal water. One major

benefit of this project is that its technology could have wide-scale application throughout watershed ecosystems. Eco technologies can be built at appropriate scales in the river or canal water, based on sediment volumes needing treatment to restore water quality; custom designs for each site can be easily scaled up or down to adapt to different conditions or needs with minimal expenditure. This technology is ideal for implantation in large-scale watershed restoration because it can be moved relatively easily.

Pleurotus ostreatus and *Trametes versicolor*, both of which can degrade petroleum hydrocarbons, were inoculated throughout the substrate. As the mycelium mass expands, the mycelium's surface area exposed to contaminated water and sediment increases. Most white rot fungi secrete extracellular enzymes that can catalyze oxidation processes via mechanisms that utilize similar pathways as *Pleurotus ostreatus* uses to oxidize PAH's.

During this project the sediment characteristics changed, thus indicating some degradation but unfortunately the project did not have enough funding to conduct a chemical analysis of the remaining sediment. Their next step is to seek additional funding so they can conduct proper analysis.

This study provided beneficial information for implementation of either direct or full-scale systems. The project has shown how technology using native organisms from all five kingdoms of life work well to remove contaminants from both water and sediment. The second round of pilot studies will seek to optimize the technology to quantify appropriate scale and treatment rate for a full-scale system. EPA has awarded the Eco-machine project another $700,000 to develop this technology.

Fourth example. *Mason County Mycofiltration Projects*. If Mason County reinitiates its prior projects, I suggest they implement mycofiltration installations at: Oakland Bay, Daniels Rd., Beaver Ave, Wiley Lane, and Eckler Rd. Four other possible sites include 1) Annas Bay area: 1/8 of a mile from the existing installation at Finch Creek, junction of Hill Creek and Hwy 101, 2) two at the junction of Main Street and Warren Drive, as well as 3) Hwy 101 between No Name Creek and Valley Rd.

One issue that this project experienced with burlap sacks is that the mycelium, at times, dies off. This issue could be resolved if mycofilters were appropriately stored when not being used. Another issue was that extra debris and sediment prevented water from flowing. I suggest using a type of screen upland from the installation to prevent leaf litter from restricting the flow of water. It is important to elevate the sacks slightly above waterways to capture contaminants. Heavy water flows have caused bags to blow out; to prevent this from happening in the future other materials to encompass the mycelium and substrate need to be explored.

We see two additional ways to improve the effectiveness of this study. First, the way the bags were being stored before installation hindered their effectiveness. Second, bags were not replaced within the ideal time periods. This method shows that there is a limited window of efficacy, but considering its effectiveness versus costs, mycofilters still appear economically feasible given the more costly alternatives.

Scientists working on this project concluded that there are some limitations to this application and that it needs to be further researched and developed before being designated as a BMP by the EPA. I believe that the suggestions I articulate above in conjunction with recommendations by Paul Stamets' team would create a more effective

mycofiltration FCB reduction database. Most mistakes that occur in these types of installations are human induced. Also, with each experience, methods will be better refined.

Fifth example. *Field Demonstration of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington.* Results of this study revealed mycofiltration reduced FCB by 90-97% when using native vegetation and natural microbial assemblages in unison, compared to the control bioretention cell without fungi (which reduced FCB by 66-73%). Battelle scientists concluded that fungi increased the effectiveness of the bioretention cell by an additional 24%. They used a bioretention cell (rain garden) as a control and a bioretention cell coupled with fungal mycelium and mycorrhizae as the treatment. Their design protocol was to see if adding the bioretention cell infused with fungal species *Pleurotus ostreatus* and mycorrhizae increased the ability of removing fecal coliform bacteria.

Biological technologies that can remove harmful toxins are undeniably needed, but many believe that their advantages are touted in an overly optimistic way. I suggest that even more biological technology efforts need to be employed to prevent pollutants from entering the environment in the first place. Stricter regulations for landowners with failing septic tanks need to be enforced by Department of Health by costly fines, if preventive measures prove unsuccessful. In addition to being fined, polluters need to be reeducated on the negative environmental impact of their practices.

Using mycorestoration in conjunction with other biological solutions for removing chemical toxins and heavy metals, i.e., synergistically rather than individually applied biological remediators to achieve ecosystem health is a promising area. I predict this

approach will become more commonly utilized because of its combined effectiveness. Scientists in this study found the bioretention cell alone decreased fecal coliform count forming units (CFU) by 66% but the bioretention cell infused with mycorrhizae was able to reduce CFU by an additional 29%, totaling 90% reduction, thus showing that adding fungi significantly enhanced the remediation methods in practice.

Pillar III: Mycoforestry

Mycoforestry is the use of fungi beneficial to trees to aid in the regeneration of forests. An example is the re-establishment of a new forest on land devastated by repetitive slash-and-burn clear cutting practices. The interaction of fungi and trees allow for healthy, sustainable fertile soils. In the following section, I focus on mycorrhizal species.

Mycorrhizal fungi have been coevolving with over ninety percent of the plants on earth through a symbiotic association, exchanging important nutrients. One single mycorrhizal fungus can help many different plant species fight off pathogens, compensate for soil nutrient loss and ameliorate the effects of drought. These associations have evolved over millions of years creating a mutuality where vital elements are shared for survival. The word "mycorrhizal" originated from the Greek words "mykes" meaning fungus, and "rhiza" meaning root (Stamets, 2005; Amaranthus, 2009). There are two major types of mycorrhizae: endomycorrhizae and ectomycorrhizae. When mycorrhizae develop in the cortical cells, resulting in swelling of roots, usually black in color, they are termed "endomycorrhizal", "endotropic", or "Vesicular-arbuscular mycorrhizae" or "VAM". After inhabiting the roots, the fungi project their filaments, known as "mycelia"

into the soil, extending the plant's roots and root absorbing capacity from approximately ten to a thousand fold, significantly more than what the plant would be able to hold alone. Mycorrhizal feeder roots give plants the ability to up-take nutrients and water from natural soils. See fig. 27: *Maple tree mycorrhizal comparisons.* The mycorrhizal fungal symbiont receives both shelter and sugars from the plant. The host plant receives phosphorus and nitrogen, and both aids the plant in times of drought and increases its salt tolerance. Overall increase in plant growth and development is achieved when mycorrhizal fungal communities are well established. These mycostructures are essential to plants, especially trees, that grow under stressful conditions (Marx et al., 1989) such as drought, sudden climate change, fire, pathogens.

Figure 27: *Maple tree mycorrhizal comparisons*

Note. Bigleaf Maple (*Acer macrophyllum*); left Maple tree without mycorrhizae, right Maple tree with mycorrhizae (Stamets, 2006).

Similarly, food crops joined with mycorrhizae can increase the effective surface absorption rate from their roots by several hundred to several thousand fold. In some cases, farmers are able to increase their crops by 30% or more (Amaranthus, 2009).

Although "Degraded Soils, Food Storage and Eating Oil" (Adinarayana, 2001) is largely focused on agricultural crops and its symbioses with mycorrhizal fungi,

mycorrhizae clearly has benefits that can be exploited in mycoremediation, mycoforestry and possibly utilized in future mycofiltration installations.

Scientists believe that it is necessary to monitor and assess long term results after contaminated soils are remediated. Mycorrhizae can be used to treat large quantities of surface contaminated soils, and in addition are low in cost. Adinarayana et al. suggest only larger scale projects can "… demonstrate the ease and viability of the inoculation methods suggested in the literature."

Plants symbiotic with mycorrhizae are known to tolerate higher temperatures, a variety of soil-based and root-borne pathogens, and increase in heavy metals. Since crops inoculated with mycorrhizae are able to better uptake nitrogen resulting in a reduction in the need for synthesized fertilizers. Fertilizers cannot maintain healthy roots, improve soil structure or aid in water up-take whereas mycorrhizae can. In fact fertilizers negatively affect the factors listed above. Fertilizers can lead to deteriorated water quality, soil structure and salinity (Amaranthus, 2009).

Even though 80% of our atmosphere is nitrogen, plants cannot uptake nitrogen in gas form; they can, however, absorb nitrogen indirectly in its form fixed by bacteria cooperating with mycorrhizal fungi. Rhizobium bacteria are a classic example. Amaranthus, a mycorhizzologist, argues that mycorrrhizal fungi can help plants take up nitrogen, without chemical fertilizers, pesticides, or extensive irrigation. The use of organic amendments and biological inoculants like mycorrhizae fungi has been widely studied and proven beneficial by several scientists. Also, the use of mycorrhizal fungi is more economically feasible than chemical soil supplements. In North America, large-scale conventional and organic farmers are utilizing mycorrhizal fungi for common food

crops, e.g., wheat, soybeans, corn and flax. In India, farmers are utilizing mycorrhizal fungi to reduce their use of chemical fertilizers by 50% without any loss in crop yield of crops. “Clearly we stand at a crossroads. We must feed the world today without destroying future generations’ ability to produce enough food. We need an approach that maximizes agricultural production while restoring clean water, protecting the environment and building soils, and sustaining soil resources.” (Amaranthus, 2009, pp.3). I highly concur with Amaranthus’s assessment on focusing upon an organic biologically based strategy for managing soils. The following section describes each type of Mycorrhizal associations.

Ectomycorrhizae. ectomycorrhizae form a structure called Hartig Net between the plant’s root cells, or cover the surface of the feeder roots (Marx, 1989). Ectomycorrhizae are a mutualistic, often obligatory, symbiosis between the hyphae of certain basidiomycetes and ascomycetes and the fine roots of certain plants. The ectomycorrhizally endowed feeder roots develop a swollen appearance and in pines they usually appear as forking habit. Ectomycorrhizae are normally found on the following tree species: alder (*Alnus*), beech (*Fagus*), Douglas fir (*Psedudotsuga menziesii*), eucalyptus (*Eucalyptus*), fir (*Abies*), hickory (Carva), oak (*Quercus*), pine (*Pinus*), and spruce (*Picea*).

Marx et al. suggest that *Thelephora terrestris* is the most common ectomycorrhizal fungus used by bare-root seedling nurseries in the United States. This mycorrhizal species is well adapted to growing conditions in nursery beds but is not equipped for adverse conditions of many reforestation sites (Marx et al, 1989). Where *Thelephora terrestris* may not be effective, another fungus, *Pisolithus tinctorius* (“Pt”), often will be. Research

showed that when tree seedlings are inoculated with Pt the number of culled seedlings is reduced while survival and growth in field planting are increased. These benefits are most apparent in adverse forestation sites such as strip-mining banks.

Vascular Endo-mycorrhizal (VAM). Endomycorrhizae are normally found on the following tree species: ash (*Fraxinus*)**,** cedar (*Thuja, Chamaecyparis*), cypress (*Cyparissus*), gum, (*Eucalyptus*)**,** maple (*Acer*)**,** poplars (e.g., Cottonwood)**,** sycamore (*Platanus*)**,** walnut (*Juglans*),and other conifers. Endo-mycorrhizal relationships also occur on agronomic crops, such as corn, sorghum, millet, sudex and grasses utilized as cover crops in tree nurseries. Such mycorrhizal crops add organic biomass to the soil while reducing water and wind erosion.

Most major hardwood species form endomycorrhizal (EM) relationships and need these for normal development in forest plantings. In nursery settings, adequate development of endomycorrhizae has two major benefits. First, seedling quality and success is improved. Second, hardwoods that have good lateral roots and endomycorrhizal development will have a higher rate of survival than without. Hardwoods armed with endomycorrhizae are more capable of competing within harsher environments and undesired vegetation on the site (Marx, 1989).

The forest floor usually lacks phosphorus in a form available to trees. Endomycorrhizae can help phosphorus uptake. Without the help of these phosphorus transporters, some trees would dwindle, sicken and likely die. Despite the fact that these mycorrhizae occur naturally in the forest, they need to be managed in nursery soils (Marx, 1989). In nursery beds, phosphorus fertilization must be closely monitored or it could

hinder mycorrhizal development. Soil phosphorus should be kept between 75-100 parts per million (ppm). If seedlings without mycorrhizae are exposed to phosphorus levels below 50 ppm, development will be hindered. If artificially applied phosphorus raises levels above 100 ppm, endomycorrhizal development will be adversely affected (Marx, 1989). The cost of one pound of standardized mycorrhizae is approximately $59.95 with a suggested rate of 1-3 teaspoons application to each plant. For example, 2 teaspoons of mycorrhizae mixture is recommended per plant for inoculating ferns.

First Example: Mycoforestry Pilot Study
Mycoforestry Research Pilot Project on Cortes Island, Canada 2003- Present

In 2003, Fungi Perfecti purchased 160 acres near Mary's Point, on Cortes Island, British Columbia, Canada, of which approximately 60 acres were clear-cut by the previous owner. Where some might have seen a devastated landscape, Paul Stamets and his partner Dusty Yao saw an opportunity for demonstrating mycoforestry strategies that would reestablish a healthy forest, ultimately exhibiting old growth characteristics.

While traditional forest practices prescribe burning the debris after clear cutting, this was an opportunity to show how other practices could be successful. This multi-lifetime experiment will compare the effectiveness of introducing mycorrhizal fungi with top-dressing; the use of mulching through chipping trees and limbs that were left after clear cutting or which came down by wind throw. Top-dressing results in delayed release nutrients and aids in retaining moisture.

In forests, terrestrial ecosystems adjacent to wetlands and riparian zones can regulate retention and the release of nutrients and carbon into the aquatic systems. Hydrochemistry is a subdivision of hydrology that deals with the chemical characteristics

of the water on and beneath the surface of the earth. Water – in all forms – is affected chemically by the materials with which it comes into contact, and it can dissolve many elements in significant quantities. Chemical hydrology is concerned with the processes involved and thus includes the study of phenomena such as the transport of salts from land to sea (by erosion of rocks and surface runoff) and from sea to land (by evaporation, cloud formation, and precipitation), and the age and origin of groundwater in desert regions and of ice sheets and glaciers. Water moving through shallow soil horizons as an important contributor to stream hydrochemistry. Studies like these provided empirical data that is utilized to determine relationships between soil or surface water chemistry and soil chemical properties, soil physical properties and terrestrial basin topographic characteristics. Analyzing these relationships can be used predict ecosystem water response and is important in identifying the influences of natural or anthropogenic factors. Understanding natural soil processes is vital to decision-making when implementing regulations [[3]].

Canada has a strict view of management and harvesting timber on land within 200 feet of waterfront. On Cortes Island, it is illegal to harvest timber without appropriate permits. On the 160 acres testing mycoforestry, due to environmental covenants, all the wood that is harvested must be acquired from natural processes like wind throw during harsh winter season. If timber should fall within a leave-zone it must be left to aid the ecological health of that habitat to allow natural succession.

Approximately 35,000 Douglas fir (*Pseudotsuga menziesii*) and western red cedar (*Thuja plicata*) seedlings were planted in November 2003. Half the trees were root-dipped

[3] http://cfs.nrcan.gc.ca/projects/118

with mycorrhizae while the other half were not; in other words, the roots of half the cedar were each covered in approximately half a million spores of the endomycorrhizal *Glomus intradices* while half the Douglas firs were each exposed to similar amount of ectomycorrhizal *Rhizopogon parksii*. In the summer of 2004, volunteers distributed a gallon of wood chips around half the spore-treated and half the non-spore-treated trees to test if decomposing woodchips further benefited tree growth.

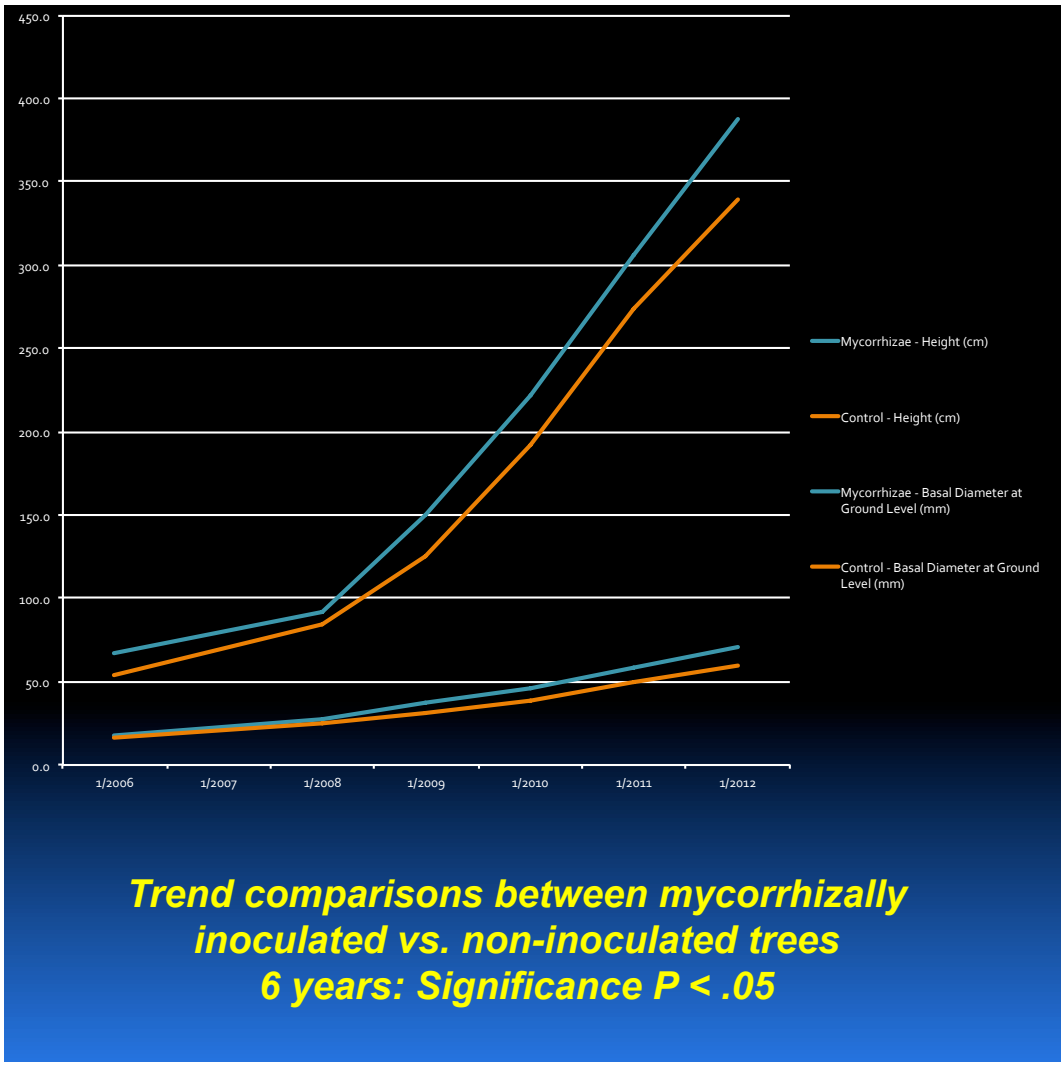
Immediate benefit received by top-dressing was the lowering of the soil temperature for newly planted seedlings. While native fungi decomposed the wood chips, a slow release of nutrients travels into rhizopshere. Mushroom mycelium de-molecularizes plant fibers (lignin and cellulose), adding healthy soil as a positive direct consequence (Stamets, 2004).

The goal of this project is to increase the soil depth sequentially resulting in greater carrying capacity for the tree and natural successions. Mulching reduces fuel load in forests while increasing moisture retention. Stamets sees wood chips as valuable ecological currency that should be re-invested into forests to enhance sustainability. There are many different interpretations and definitions of sustainability, so it is important to define this term to eliminate confusion. Here, sustainability is the characteristic by which a process or state can be indefinitely maintained to meet or exceed a certain level whose metrics included increased timber mass, biodiversity, humus accumulation, aquifer protection and hydrological dynamics.

In September of 2004, 700 trees were tagged and measured for height and girth. The data initially revealed a net increase of 9-10% in both height and girth for 10-month duration. Annual measurement will be gathered, and after 10 years a chi square data

analysis will be completed. At 8 years, statistical analysis showed significance (P<.05) in the mycorrhizally treated trees increasing in biomass (as measured by height and girth) over non-mycorrhizally treated trees.

Figure 28: *Trend Comparisons between Trees inoculated with Mycorrhizae vs. non-inoculated trees*



Note. This chart shows comparisons between trees that are inoculated with Mycorrhizae compared to those trees that were not inoculated with mycorrhizae.

In the summer of 2011, I visited the site and observed healthy trees with approximate heights averaging 9-10 feet; where there was once a scared devastated forest

is now an oasis of life, with increasing species biodiversity as a new ecological community emerges. As of December 2012, the mycoforestry project continues requiring low maintenance, periodically requiring the Vemar cones to be removed once the trees are above the browsing height of deer.

Logging companies could follow this application by chipping woody debris and leaving them within the cut forestland, dispersing the nutrient feedstock over the landscape to help refuel the carbon cycle. Chipping will add to cost in short term but the long-term benefits from mycologically enhanced forests will result in ecological health and economic returns.

Approximately half of Canada's land surface is covered in trees: 400 million hectares from coast to coast. The benefits provided by this natural wealth extend to ecological, economic, recreational, social, cultural, traditional, as well as spiritual. Canada's federal government has made caring for its forests a top priority [[4]]. The criteria and indicators for Canada's framework include biological diversity, ecosystem condition and productivity, soil and water health, role in global ecological cycles, economic and social benefits and society's responsibility.

Canada's forests purify water, stabilize soil and cycle nutrients, creating habitat for wildlife, as well as nurturing environments rich biological diversity. About 77% of Canada forests are publicly owned, while 16 % are federally owned and only 7% privately owned. Federally owed land can be managed better because of recourses and policy guidelines, therefore they have more control over protecting the land. Canada sustains a national-wide forest products industry, which supports hundreds of thousands of jobs and

[4] http://cfs.nrcan.gc.ca

contributes billions of dollars to the country's economy. Canada is committed to Sustainable Forest Management (SFM).

Critique of Mycoforestry Study

The following section is aimed to give my personal critique of mycoforestry reference to the pilot project discussed in detail in the above section.

First example. *Mycoforestry Research Pilot Project on Cortes Island, Canada.* Although within the last decade copious data have been collected from this particular site, the data have not yet been fully quantified into a usable form for data analysis. The next step for scientists working on this project is to complete the measurements and then analyze results for statistical significance after a decade of data collection. However, when conducting a study like this in the field, it is hard to determine if the trees received beneficial mycorrhizae naturally from the soil or from inoculation. I suggest before starting a field study like this that several soil samples be analyzed under microscopy in specific locations where experiments will take place. This way soil samples can be compared later in the study to determine if they naturally occurred or were introduced by scientists. We can analyze trees introduced with and without mycorrhizae in a controlled indoor setting but once in the natural environment, is much harder to distinguish introduced from native mycorrhizae. Since data for establishing significance do not necessarily need to include hundreds of trees, I recommend comparing the trees that were planted directly onto logging roads, where native mycorrhizae would be absent or extremely low. Comparing trees placed into these mineral soils, lacking humus and native mycorrhizae, could give significant results without a great expenditure of time and labor.

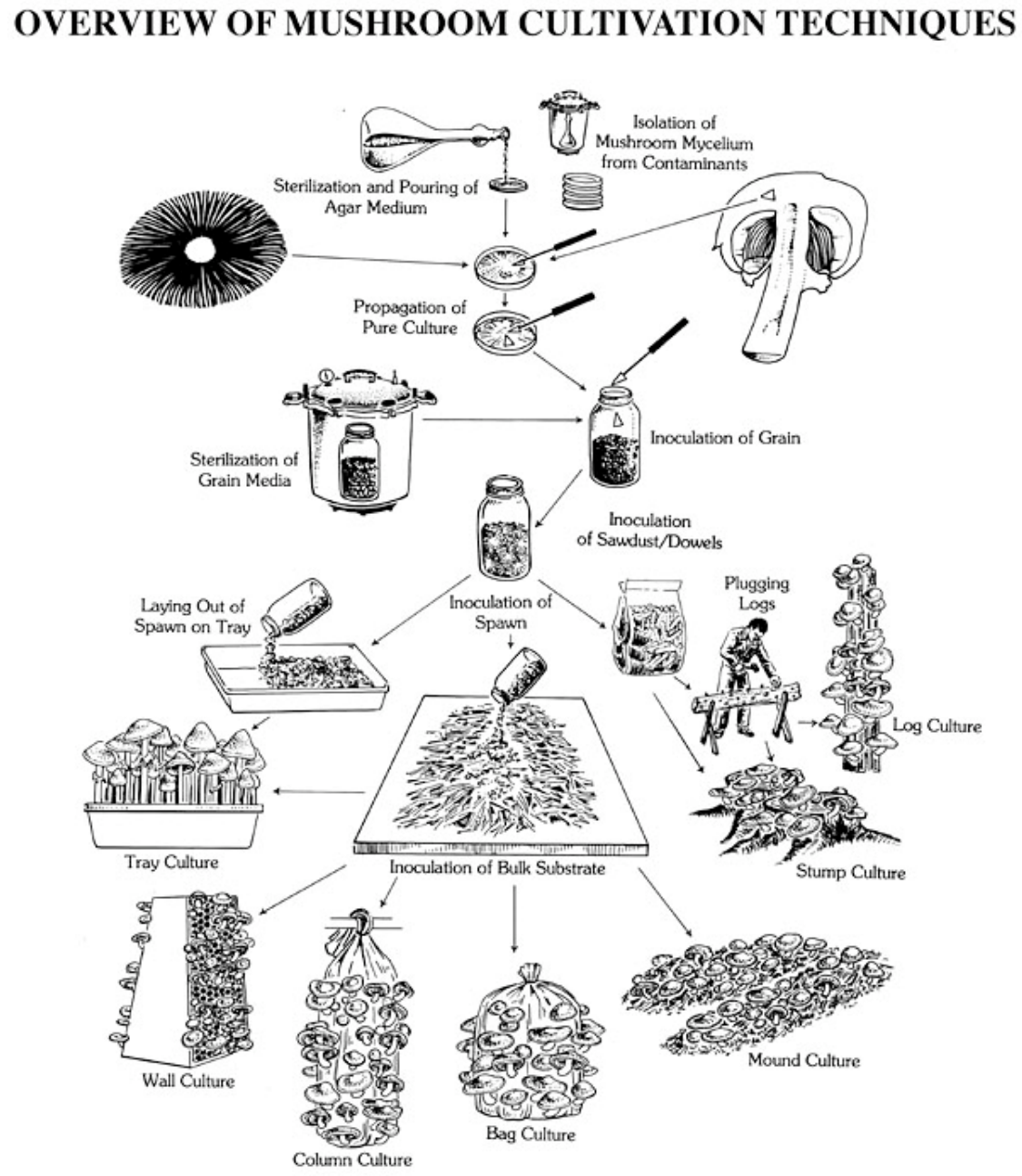
Part II
Methodology for Individual Landowner Mycorestoration Projects

High and Low Technology for Cultivation Techniques

This section is focused on high-tech and low-tech methods for cultivation; its purpose is to offer information that can help you generate mycelium spawn and implement mycorestoration installation.

The process by which mushrooms are harvested from nature and then cultivated in the laboratory (fig. 29: *Process of mushroom cultivation techniques*) is important but it is not critical to fully understand it for implementing mycorestoration at the grass roots level. If you harvest from nature keep in mind the concept of "sustainable harvest": only take a few mature mushrooms and don't devastate the population by over harvesting or taking the under developed mushrooms. The Precautionary Principle should be applied through research and application of mycorestoration, emphasizing that new methods are carefully considered before implementation to prevent collateral damage to other components essential for environmental equilibrium and health. The Precautionary Principle states that it may be better to avoid taking a particular action if there is a possibility of causing unexpected harm; a classic example here in the PNW was choosing to introduce a non-native species like scotch broom to promote slope stability, which is now considered an invasive weed, requiring laborious efforts for eradication.

Figure 29: *Process of Mushroom Cultivation Techniques*



Note. In this simplified illustration you can follow the process of cultivating mushrooms in lab setting. This process to expand your mycelium with substrates results in variety of different end products. © Property of Paul Stamets

High-tech, in vitro propagation methods of growing mycelium to create spawn is fully covered in-depth in several books such as *The Mushroom Cultivator* by Paul Stamets (1983) and *Growing Gourmet and Medicinal Mushrooms* by Paul Stamets (1997). The end-user will be purchasing "spawn", i.e., mycelium grown out on a carrier substrate such as grain, woodchips, sawdust, etc., from a reputable, and preferably local, certified organic spawn producing company. The section that follows illustrates how use the spawn, once acquired.

I will explain low technology which most of you will be utilizing. However this section is not intended to teach you how to cultivate mushrooms – for more information about cultivating fungi please refer to the aforementioned books. This part of the guide is focused on how to implement mycorestoration that can be utilized on small-scale projects. You have two convenient avenues or pathways to achieve mycofiltration installation. The easiest is purchase mycofilter bags already made and inoculated with mycelium (see Fig 30: *Mycofilters)*. The second is to make your own mycobags by expanding your commercially acquired spawn.

Figure 30: *Mycofilters*

a.)

b.)

Note. a.) New Mycofilters b.) Blake Westman holding colonized mycofilters made for the EPA grant pilot project. Photos by Paul Stamets 2012.

Once you have your mycelium for mycofilter bags or for mycofilter sheet-mulches, chose a method of grounding the mycelium in a tributary, or for soil remediation chose a parallel sheet inoculation approach also called ‘lasagna layering’ technique. Below

is a diagram that may be helpful for anchoring sacks. Also refer to Mycofiltration Project Installation fig. 31: *Four ways to inoculate burlap sacks with fungi*. This simplified illustration shows four different ways of that the installation of anchoring burlap sacks can also inoculate the sacks with specific species of fungi: galvanized nails and stakes, stakes through bags, stakes and twine, bags installed opposite each other with galvanized nails and stakes. As water percolates through the burlap sacks, mycelium breaks down toxins.

Figure 31: *Four Ways to Inoculate Burlap Sacks with Fungi*

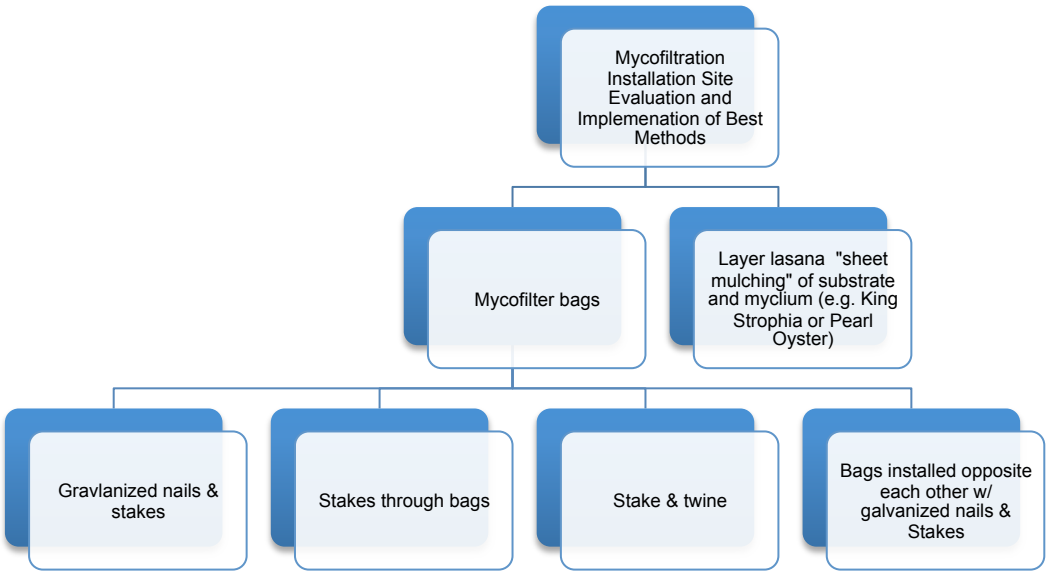
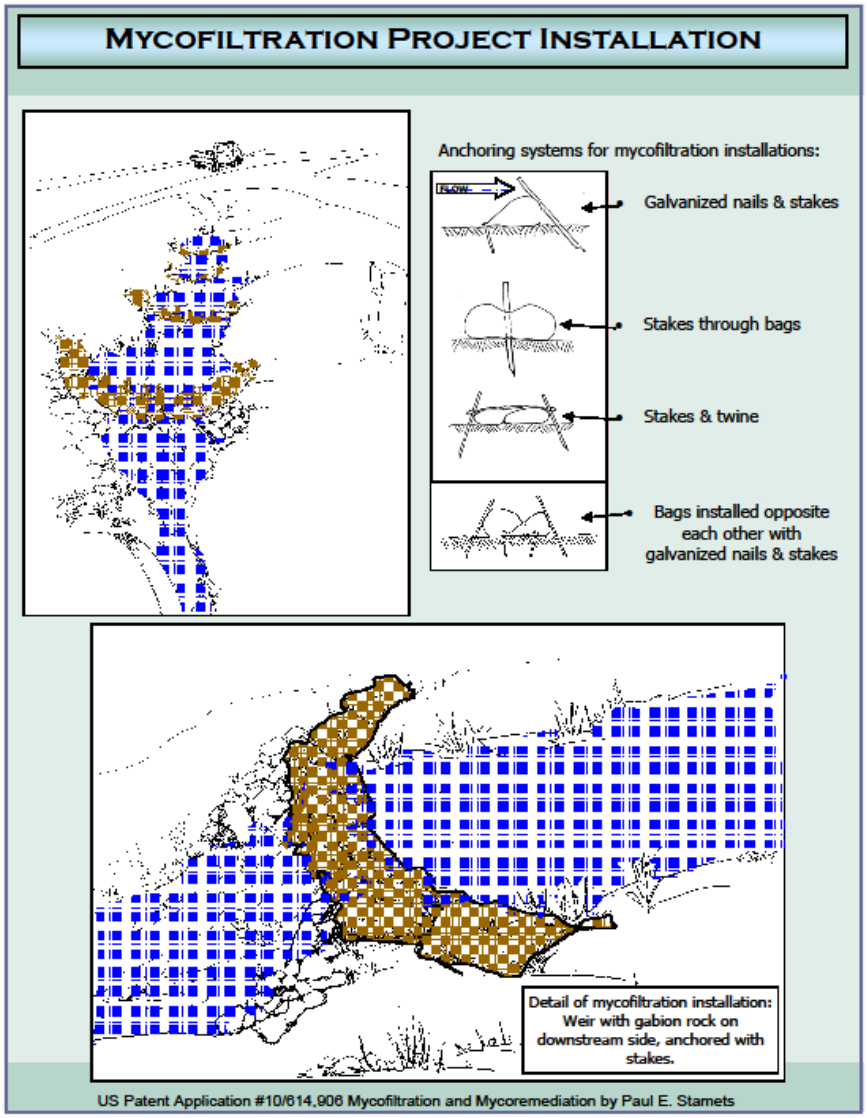


Figure 16: *Mycofiltration Symantec Installation*



Note. Illustrations of anchoring systems utilized for Mycofiltration installation within tributary. Property of Paul Stamets.

For an overview of higher technology methods for mycelial spawn production, see *Growing Gourmet & Medicinal Mushrooms* or *The Mushroom Cultivator.* These books can guide you to successful cultivation and eliminate the need to purchase mycofilters.

A synopsis of essential steps for spawn production are listed here:

1. Preparation and pouring of agar media into petri dishes.
2. Germination of spore and isolation of pure mushroom mycelium.
3. Expansion of mycelial mass on agar media.
4. Preparation of grain media.
5. Inoculation of grain media with pure mycelium grown on agar media.
6. Incubation of inoculated grain media (spawn).
7. Laying out grain spawn onto trays, or inoculation of grain spawn into bulk substrates.
8. Casing – covering substrate with a moist mixture of peat and other materials.
9. Initiation – lowering temperature, increasing humidity to 95%, increasing air circulation, decreasing carbon dioxide and or light levels.
10. Cropping – maintaining temperature, lowering humidity to 85-92%, maintaining air circulation, carbon dioxide and/or light levels.

Raw material that can be utilized from agriculture or the forestry industry to create "fruiting substrate" are listed below:

- Wood waste paper products
- Cereal straws and grain hulls
- Coconut fibers
- Corncobs
- Coffee plants and waste (e.g. used coffee grounds)
- Tea leaves
- Sugarcane bagasse
- Bananas fronds
- Seed hulls (cotton seeds, sunflower seeds, and oil-rich seeds)
- Almond, walnuts, pecan, peanut hulls
- Soybean meal, roughage (Okara), soy waste
- Artichoke waste
- Cactus waste: saguaro and prickly pear, yucca, agave

Cost of mycelium is ~ $125-325 per test-tube, depending on the species. One could eliminate this cost by harvesting species from the forest using sustainable harvesting principles. If you would like to expand your mycelium to make mycofilter or mix in mycoremediation in situ, below is a checklist that includes all the material one should consider:

High-Technology Check-list

1. Autoclave
2. Petri-dishes
3. Para-film
4. Glass jars
5. Malt agar
6. Micron (HEPA) filtered laminar flow bench
7. Digital thermohygrometer
8. Synthetic filter disks
9. Scalpels
10. Bacticinerator® electric scalpels sterilizers
11. Spawn bags with filter patch
12. Impulse sealer
13. Erlenmeyer media flasks
14. Cost of electricity
15. High-sensitive digital scale
16. Organic rye
17. Space and time in lab.
18. Labor: Skilled Lab technicians & educated scientist

Low-Technology check-list expanding medium (for individual landowners):

1. Spawn from mycologist
2. Cardboard
3. Hydrogen peroxide (0.3%)
4. Zip-lock plastic bag
5. Medium (alder woodchips, straw, rice, ect.)
6. Burlap sacks (non petroleum treated)
7. Labor
8. Soaking tank

For constructing mycofilters you will need the following:

1. Substrate (alder wood, chips, sawdust, straw ect.)
2. Tools (shovels, pitch fork)
3. Rubbing alcohol

4. Burlap sacks
5. Twine or hemp string
6. Plastic bags
7. Rent or borrow a tractor if you don't have one.
8. Wooden stakes

After sending soil or/and water samples to a local environmental toxicity lab for analysis and receiving the results, the landowner or stakeholder will complete the 2012 Mycorestoration Site Description (refer to fig. 32: *2012 Mycorestoration Site Description* at the end of this section) and identify which toxins and heavy metals are present on the land. After careful analysis of the many parameters of concern, he or she will then choose a fungal species that has the ability to ameliorate the identified chemical toxins or accumulated heavy metals of concern. Please refer back to Figures 5: **Mushrooms with Activity Against Chemical Toxins** (page 21) and Figure 6: **Mushroom Species' concentrations of Heavy Metals** (page 22). The following section explains low-tech methods of implementation of mycoremediation and mycofiltration projects.

The simplest method for making a substrate like woodchips or straw ready for the introduction of spawn is ambient temperature fermentation. This method, although now readily articulated, was only recently refined for use in mycoremediation and reduces costs by several orders of magnitude because high heat pressure sterilization, the use of caustic chemicals, or much of the traditional infrastructure for incubation of mycelium are now deemed unnecessary.

Simplified Method for Generating Mycoremediation or -filtration Mycelium

STEP 1: Acquire freshly chipped hardwood or conifer (not fungally resistant tree species like cedar, walnut, or redwood).

STEP 2: Place wood chips and/or straw into containment vessel such as an open tank, farm watering trough, seafood "tote" or plastic lined pond.

STEP 3: Fill container with wood chips until submerged with fresh or salt water at ambient temperature, preferably 40-60^0 F. Cold fermentation of wood chips could be utilized (see fig. 33: *Ambient Temperature (40-60 F.) Fermentation of Woodchips in Fresh Water*). Cold fermentation works because an anaerobic environment with multiple species of bacteria Klebsiella followed by exposure to oxygen kills anaerobic microflora thus the substrate exposable by the oxygen loving mushroom mycelium.

STEP 4: Allow the container to sit undisturbed for 1-3 weeks. A biofilm will form and fine gaseous bubbles will be emitted after a few days.

STEP 5: Drain container completely.

STEP 6: Wait ~2-4 hours then inoculate with 2-10% spawn by volume.

STEP 7a: Allow spawn to incubate within the container where soaking occurred.

STEP 7b: Fill burlap sacks with inoculated mycelium (optional if doing mycofiltration).

STEP 8: Place into incubation environment at 50-70^0 F for one week. Lower temperature to 35-55^0 F for an additional 4-8 weeks.

STEP 9: Move myceliated substrate to mycoremediation site.

STEP 10: Position myceliated substrate for optimal configuration according to site characteristics.

Figure 33: *Ambient Temperature (40-60 F.) Fermentation of Woodchips in Fresh Water*

Note. This photograph shows the fermentation process of wood chips before they are used to create mycofilters. Barrels are used to keep substrate submerged. Photo by Paul Stamets, 2012.

Depending on contaminant load characteristics, slope, weather, and other environmental factors, mycofilters may have to be renewed for optimal performance based on results determined by periodic-testing. Mycofilters need to be installed at a weather-appropriate time, since fungi can tolerate colder temperatures better than hot temperatures. Such care constitutes being sensitive to the biological cycles and environmental conditions mushrooms thrive in. Good luck to you and your mycorestoration endeavors!

The below photographs are examples of small-scale mycofiltration installations in Washington State. Refer to fig.34: *Small-scale Mycofiltration Installation Examples*

Figure 34: *Small-scale Mycofiltration Installation Examples in Washington State*

a.)

Note. Small-scale mycofiltration installation on Oyster Bay Road at Pat Labine's farm, Thurston County, Washington. Photos a-c by Paul Stamets

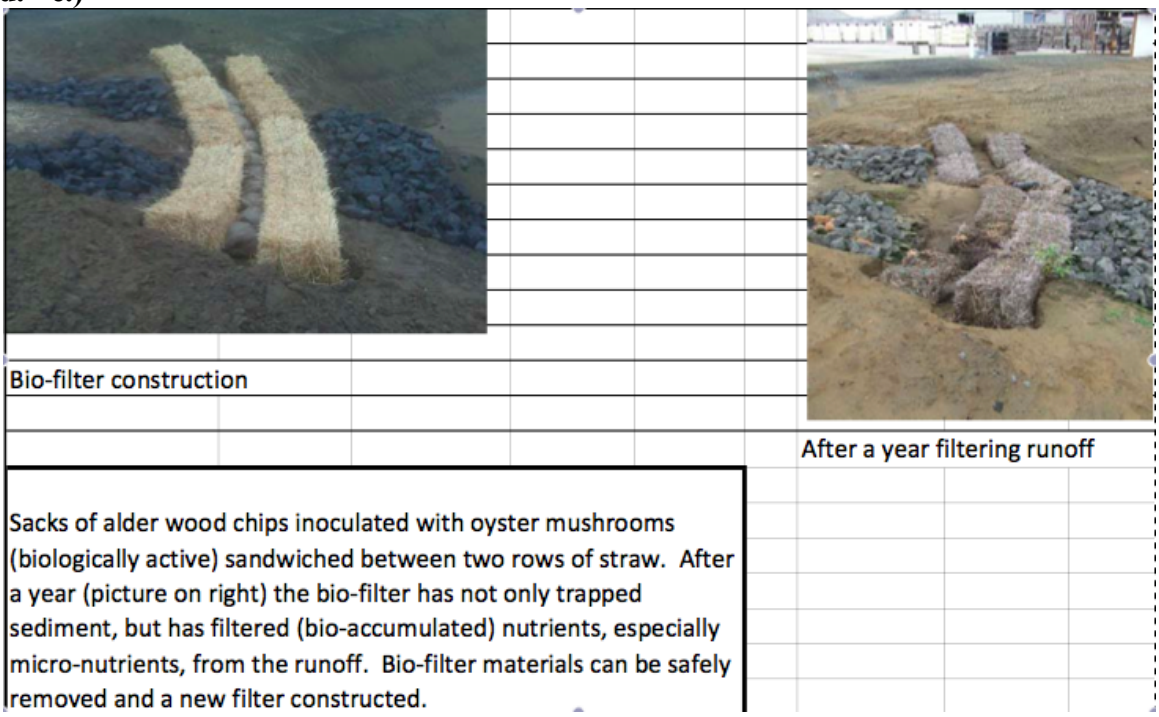
b.)

Note. Small-scale mycofiltration installation on Oyster Bay Road at Pat Labine's farm.

c.)

Note. LaDena Stamets with triangular mycofiltration mycobag design. a-c Photographs by Paul Stamets

d. - e.)



Note. Small-scale mycofiltration installation example, at DNR Webster Tree Nursery, Washington State. Photo by John Trobaugh

Figure XX: *2011 Mycoremediation Site Description*

FUNGI PERFECTI, LLC

PHONE: (360) 426-9292 • FAX: (360) 426-9377
EMAIL: info@fungi.com •
(Please fax us this form)

2011 MYCOREMEDIATION SITE DESCRIPTION

ver.2.4

This site description outline covers many, but not all variables, which are necessary for Fungi Perfecti, LLC to evaluate using fungi to remediate a contaminated or toxin-threatened property. Please answer each field by checking the appropriate box and fill out the blank fields. List features specific to your needs. Add additional comments, if necessary. We will, on a case-by-case basis, decide whether or not the skill sets of our consultants and resources can address your needs. A $ 200 non-refundable application fee is required for each site evaluation. We will respond with our comments within 30 days.

Submitter		**CoSubmitter**	
Address		**Address**	
City, County, State, Zip		**City, County, State, Zip**	
Phone		**Phone**	
Email		**Email**	

Project Title and Site Name : Address of Site	GPS Coordinates and/or Google Earth/Virtual Earth URL:	Current Tax Assessed Value of Property: Year of Most Recent Assessment:

Biography of Submitter	☐ I am financially responsible for paying for the mycoremediation tests and applications ☐ I am not financially responsible for paying for the mycoremediation tests and applications I am ☐ or I am not ☐ the only person making the decision about the course of remediation. The third party who is ☐ or will be ☐ responsible is:
Biography of Co-submitter	☐ I am financially responsible paying for the mycoremediation tests and applications I am ☐ or I am not ☐ the only person making the decision about the course of remediation.

Please mark appropriate categories:

Toxins localized on subject property? Yes ☐ No ☐ Toxins coming from off-property to subject property ? Yes ☐ No ☐ Source of toxins known ? Yes ☐ No ☐
Source:

Land Ownership Description	☐ We are the owners	☐ We are consultants	☐ We are for-profit	☐ We are non-profit
Legal Description of Subject Property				

☐ We are not yet funded for this project ☐ We are currently funded for this project for $ for the time period of: _____ from: _____
Funding Entity:

Site Description ("Ecologicals")

The property is best described as having these combinations of characteristics

- ☐ wetlands
- ☐ forested
- ☐ drylands
- ☐ farm
- ☐ residential
- ☐ city
- ☐ industrial
- ☐ multi-use
- ☐ public
- ☐ private
- ☐ under covenants
- ☐ not under covenants

Soil Characteristics at
Surface:
2 ft: _____
4 ft: _____
8 ft. _____

Water Table Depth:

Nearest Well to Toxic Site:

Adjacent Properties are:

- ☐ Also sources of pollution
- ☐ Sensitive Ecosystems

- ☐ Underlayment impervious
- ☐ Underlayment pervious

Government Agency Involvement

- ☐ government agency supervising cleanup
- ☐ EPA – Federal
- ☐ EPA – or Dept. of Ecology equivalent –State

Contact Person at Agency:
Position:
Address:

Phone Number:
Email address:
Website:

- ☐ Please contact this person
- ☐ Please do not contact this person

- ☐ No government agency currently involved
- ☐ Private organization besides landowner is involved.

Name:

☐ Is this property currently in legal litigation or threatened to become involved in litigation?

If so, by whom:

Contact Person at Agency:
Position:
Address:

Phone Number:
Email address:
Website:

Stated Cause:

Microbial Contaminants

☐ Coliform bacteria _____cfu/gram ☐ Non-coliform bacteria _____ cfu/gram

Heavy Metals

☐ Mercury _____ppm ☐ Cadmium _____ppm ☐ Lead _____ppm ☐ Copper _____ ppm ☐ Arsenic _____ ppm

Chemotoxins:

☐ Pesticides: _____ ☐ Herbicides: _____ ☐ Fungicides: _____ ☐ Oil related: _____
____ ppm ____ ppm ____ ppm ____ ppm

☐ Endocrine Disruptors _____ ☐ None of the above: The toxins of concern are: _____
____ ppm ____ ppm

To What Depths Have Toxins Been Found ? Special Considerations:

Uniformity of Toxins in Landscape: ☐ Uniform ☐ Irregular

Point Source(s) of Pollution: ☐ Multiple Loci ☐ Don't know

☐ Sloped %_____ ☐ Flat ☐ Contoured

☐ Analyses of above was done by 3rd parties. Name and Address of Analytical Laboratory: Phone Number: Website:

Analytical report is available for our review? ☐ Yes ☐ No

Have any native fungi been observed on the property: ☐ **Yes** ☐ **No** **Identification:** by whom: **Photos taken:** ☐ **Yes** ☐ **No**

Spores Collected ? ☐ **Yes** ☐ **No** Cultures obtained ? ☐ **Yes** ☐ **No** Specimens obtained ? ☐ **Yes** ☐ **No**

I attest the above statements are true and factually accurate, to the best of my knowledge.

Signed by: _____ of _____(Organization) Date: _____WE

WE DO NOT ACCEPT SAMPLES OF TOXIC WASTES AT OUR FACILITY. DO NOT SEND ANY MATERIALS WITHOUT PRIOR WRITTEN APPROVAL

Please use this space for additional comments:

Note. Site Description outline helps property owners qualify the site they are considering using mycorestoration. © Paul Stamets 2011.

Part III
Conclusion and Future Research

This thesis has documented the ability of mycorestoration to counteract anthropogenic pollutants the environment. Such pollutants cause degradation to human health as well as to other natural systems, ultimately increasing the cost of living in these ecosystems. Using mycorestoration increases the inherent sustainability of habitats, reducing the need for remedial practices while fortifying the ecological benefits healthy habitats provide: clean water, clean food, clean air, and healthy inhabitants.

Mycoremediation and mycofiltration are low-cost, time conservative, low maintenance biological solutions, which decrease toxicity in soil better than other methods. Moreover, the cost of removing petroleum hydrocarbons using mycoremediation techniques is significantly less than other methods such as soil washing and bioremediation.

Many attempts have been made to develop methods to remove oil spills and toxins from water and soils. Mycoremediation and mycofiltration are worthwhile techniques for reducing petroleum-based pollutants in our environment. Research has shown mycoremediation can remove hydrocarbons such as oil, petroleum based products, pharmaceuticals, pesticides, and farm wastewater. For example, TAH reduction highlighted in this thesis averaged 95.5%. In most cases reduction of PAHs was 86-92%. FCB reductions averaged 92%, with the highest reduction being 97%. Through my literature review, I found that reductions in contamination levels of fecal coliform bacteria **(**FCB**)** ranged from 87 to 97%, while polycyclic aromatic hydrocarbons (PAHs) reduction ranged from 57 to 97%. Total aromatic hydrocarbons (TAH) reductions were 91 to 99%. Mycoremediation scientists agree more research needs to be conducted to

further our understanding of species that will be the most effective against specific toxins. Species specificity factors are critical to match before executing installation for achieving the best results.

While there are hundreds if not thousands of enzymes secreted by fungi, many of which can break down PAHs, only a few have been thoroughly researched for the purposes of mycoremediation. Since so many fungi have not yet been thoroughly tested for enzymes, there are opportunities for future research to discover new enzymatic systems that can metabolize contaminants detrimental to ecosystem health. Manganese-dependent peroxidase enzymes in particular are strong catalysts of oxidation processes. Fungi excrete metal-binding and up-take metabolites associated with complexolysis or ligand-promoted dissolution, including carboxylic acids, amino acids, siderophores and phenolic compounds.

Clearly, mycorestoration can be documented as effective under some circumstances, but the range of applicability needs to be expanded. By building upon the synergistic relationships fungi have in nature, mycorestoration applications can be become more effective and have a wider range of applicability.

Increasing awareness for environmental scientists and public policy makers is essential. Policies and funding limitations have been major obstacles that have prevented the use of wide spread mycoremediation to clean up of chemical toxins. Research and publications about mycorestoration are available and continue to compile as does public interest. Politicians and other environmental sectors should push to make this innovation available globally for the health of our environment that directly affects our well-being. The US EPA is investing tens of thousands of dollars in mycorestoration grants. Once

mycorestoration is adopted as a Best Management Practice (BMP), these applications can be commercialized and utilized for global contamination clean up of a variety of chemical toxins. Toxins and pollution know no borders and what we do affects our neighbors locally and internationally. For example, a paper mill in Tacoma, Washington, caused high levels of arsenic in contaminated soils on Vashon Island. Such situations could use mycorestoration techniques but landowners usually don't know how to get started. Future research will emphasize the best practices for implementation of mycoremediation, mycofiltration and mycoforestry methods as well as applicability to site specificities.

Biological systems are inherently different from mechanical systems. Given this, we need to work within the biorhythms of ecosystems. The number of individuals or biomass of a species that an ecosystem can support is known as its carrying capacity. Reducing our ecological footprint, which is the influence that people's patterns of consumption and life style has on the surrounding ecosystem and across the globe, enables us to live within the carrying capacity of the environments that support us. My intention of this thesis has been to bridge the gap between government funded research studies and individual landowners with small-scale installations, with the goal of empowering landowners to take control and protect their land from chemical toxins and heavy metals. This thesis has defined the parameters for designing a private, individual site-specific mycorestoration installation. Projects with the Makah First Peoples, the Squaxin First Peoples, the Mason County Departments of Health, the Washington State Department of Transportation (WSDOT), and private companies such as Ridolfi, Inc., and Ecological Design, Inc., help build awareness and develop data that will illustrate proof-of -concept.

This collective effort will provide the basis for more funding to demonstrate that mycorestoration is a low-cost method practice for cleaning up pollutants.

Mycoremediation and bioremediation eliminate the cost of removing thousands of tons of soil from toxic waste storage sites. Costs for storing toxic waste is expensive and continues indeterminately – much toxic waste must be guarded in a secure area for the unforeseeable future, incurring an unpredictable expense for future generations. Some toxic waste will take thousands of years to degrade. Consequently policies that allow burning, hauling, and burying of toxic waste leave the environment unhealthy and unable to achieve a healthy ecological equilibrium. A natural myco-biological solution can turn contaminated soils into useable medium for soil generation and landscaping.

Further research needs to be conducted to see what other fungal species will prove useful for mycoremediation. For instance, *Marasmiellus candidus* sensu lato is a dominant white rot species proliferating where cane berries thrive, and could be utilized for mycoremediation to clean up environments characterized by overgrowth of blackberries, salmonberries, etc., as suggested in this thesis for implementation on Tatoosh Island for PAH clean up. Another esoteric species is the recently discovered underwater mushroom *Psathyrella aquatica* found in fresh water river systems. If this species has the potential to break down toxins like PAHs or capture pathogenic bacteria it could aid in recovering fresh water environments from anthropogenic contamination.

Throughout the literature research I conducted there has been general agreement among the scientific community that there are many advantages to mycoremediation and mycofiltration: these are effective, low cost, biological solutions that require minimal maintenance and are flexible for installation in a variety of sites. That said, mycofiltration

has some limitations: First, scientists need to find stronger netting/mesh material to hold the substrates together and won't decompose before fungi have completed their life cycles or blow out from heavy water flows. Running parallel controls in several locations along each site would eliminate data errors and provide comparison of contamination levels. Another issue that needs to be addressed is using different species in interspersing mycobags; data could be collected specific to the ability of one fungal species to another. Currently, the oyster mushroom, in particular the *Pleurotus ostreatus* strain from Nisqually Delta, has become the primary species for mycofiltration installations within Mason County, Washington, but it is possible that other resilient species are more appropriate for other sites. Keep this in mind while selecting a fungal species for remediation.

Future funding and research needs to be focused on testing applications of myco-enhanced constructed wetlands, buffer zones, mycobooms and in-line wastewater treatment systems in urban and rural areas. More studies like those using eco-machines at the Fisherville project, which take a multi-kingdom approach, hold great promise. Dr. Susan Thomas' Dungeness Watershed project was helpful in understanding fungi's ability to metabolize *E. coli*.

Fungi Perfecti's EPA Bench Study "Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Stormwater" identifies which combinations of substrate and fungal species are the best for treating stormwater. The EPA grant "Mycofiltration in Urban Landscapes" will tentatively enter phase II in June 2012 with field applications at numerous locations.

Mycoforestry is another new concept showing promise. The interaction of fungi

and trees allows for healthy fertile forests and regenerating soils. Mycorrhizal fungi have been coevolving with over ninety percent of the plants on earth through a symbiotic association, exchanging important nutrients. Even though it is well known the presence of mycodiversity in soils is an indicator that soils are healthy, fungal strategies to restore devastated forests are not commonly applied. However, government agency sites, like the Washington State Department of Natural Resources at Webster's Nursery, use mycorrhizal species every few years to help establish tree saplings and improve out-planting. Mycoforestry is not just about applying mycorrhizae to one tree. Vast fungal networks of fungi link trees together by bridging root systems through hyphae, exchanging nutrients with each other, and helping assure habitat health through biodiversity support.

One single mycorrhizal fungus can aid many different plant species to fight off pathogens, compensate for soil nutrients loss and ameliorate the effects of drought. The fungi project mycelia into the soil, extending the plant's roots and root absorbing capacity many fold, significantly more than what the plant would achieve alone. Crops with mycorrhizae present can increase the effective surface absorption area of the roots by several hundreds to several thousands fold. Commercialization of methods for applications is being developed as demand increases. Adinarayana et al. (2001) suggest that only larger scale projects can "… demonstrate the ease and viability of the inoculation methods suggested in the literature."

Mycorrhizae can maintain healthy roots, improve soil structure and aid in water up-take whereas fertilizers cannot. In fact, fertilizers negatively affect the factors listed above, often leading to deteriorated water quality, soil structure and salinity. The use of organic amendments and biological inoculants like mycorrhizal fungi has been widely

studied and proven effective by dozens of universities. Also mycorrhizal applications are economically feasible at a fraction of the cost of chemical soil supplements. In India, some farmers utilize mycorrhizal fungi to reduce their use of chemical fertilizers by 50% without any production loss.

In nursery settings, endomycorrhizae have two major benefits. First, seedling quality and success is improved. Second, hardwoods that have good lateral roots and endomycorrhizal development will have a higher success of survival than without. Hardwoods enhanced with endomycorrhizae are more capable of competing with undesired vegetation and/or in harsher environments. Forest floors are usually lacking phosphorus in a form that is available to trees. Endomycorrhizae can naturally help in phosphorus uptake. Without these mycohelpers, the trees wood die. However, these mycorrhizae need to be managed in 'constructed' nursery soils which are often chemically treated to reduce possible pathogens, and in doing so, the beneficial fungi are also adversely affected.

Canada's sustainable forest practices should be echoed across the globe. Future research should explore implementation of large-scale field studies of using mycorrhizae to regenerate devastated forests. However, scientific comparisons of forests planted with and without introduced mycorrhizae are difficult experiments to manage because of naturally occurring mycorrhizae in soils and the fact that mycorrhizal species easily distribute themselves via spore transportation.

Planted Plant Production is a company created to produce a product called "Life Box." A Life Box is a corrugated cardboard box infused with mycorrhizal fungi and the seeds of several different tree species. The idea is that people receive a Life Box through

the mail then germinate the seeds contained in the box, and then plant the resulting trees to regreen the Earth. This innovative technology is aimed at sequestering carbon from the environment. Once Life Box trees are planted one can use a hand held device application to geographically pinpoint the GPS coordinates where one's trees are and calculate how much carbon has been sequestered.

Traditional methods for removing oil spills from water, e.g., skimmers, booms, dispersants, and controlled burning, are not effective. The Gulf of Mexico's April 20, 2010, event where the BP Deepwater Horizon oil rig blew up in the Gulf of Mexico resulted in the death of eleven people and the dumping of more that 200 million gallons of oil into the ocean. Only 8% of the oil was recovered. In 2011, Exxon Mobil pipeline ruptured, causing 42,000 gallons of oil to spill into the Yellowstone River that runs through one of our national parks. In 2011, the Fukushima nuclear power plant disaster was yet another example of an environmental catastrophe where mycoremediation methods might be employed.

Future work in mycorestoration will likely focus on investigating other white-rot fungi for remediation abilities – including those that could be used to counteract environmental catastrophes such as those mentioned above. I suggest more development needs to address mycofiltration site design by creating and compiling mycorestoration sites using a Geographic Information System (GIS) map showing locations of projects, including identifying toxins and contamination levels before and after mycorestoration is applied. A mycorestoration "stamp of approval" from credible and experienced mycologists could certify that sites are being managed appropriately. Further work could

create a multi-volume guide to Best Mycorestoration Practices specific to each eco-region to induce widespread pollution cleanup.

Glossary of Key Mycorestoration Terms

anthropogenic	Caused by humans.
abiotic	Non-living.
aerobic	Living or occurring only in the presence of oxygen.
Best Management Practice (BMP)	Proactive and often voluntary forest stewardship practices that have been determined to be the most effective, practical means of preventing or reducing soil and other pollutants from entering any water; streams ponds, lakes, wetlands, ect.
bioindicator	Biological indicators are species used to monitor the health of an environment or ecosystem. They are any biological species or group of species whose function, population, or status can be used to determine ecosystem or environmental integrity.
biomagnifications	process where toxins become more concentrated in animals higher levels in the food chain.
biomass	The total weight of living material in a place often expressed as weight per unit.
bioswale	Use vegetation and gentle gradients to slow and infiltrate water. Common for holding storm water.
bioventing	Bioventing is an in situ remediation technology that utilizes microorganisms to biodegrade organic constituents absorbed within the ground water. Bioventing will enhance the activity of native bacteria as well as simulates natural in situ biodegradation of hydrocarbons by inducing air or oxygen flow.
biotic	Of or having to do with life or living organisms. Produced or caused by living organisms.
bole	A trunk or main stem of a tree.
brown rot	A condition caused by the degradation of cellulose by fungi. It leaves the substrate a brown color largely due to undecomposed lignin. Soild blocks of wood are used for testing wheather a dungus causes brown rot or white rot.
carrying capacity	The number of individuals or biomass of a species that an ecosystem can support.

conifer	A cone-bearing tree with needles (e.g. pine, spruce, fir, larch).
deciduous	A tree that loses its leaves or needles during the fall and winter (e.g. Maple, Alder and Oak).
dilution	The process of making a weaker or less concentrated.
ecological footprint	The influence that people's patterns of consumption and life style have on the surrounding ecosystem and across the globe.
ecological restoration	Altering a site to reestablish the original ecosystem.
ecosystem diversity	The different biological communities and their associations with the chemical and physical environment.
ecosystem function	The interactions between organisms and the physical environment, such as nutrient cycling, soil development, water budgeting, and flammability.
ecotone	A transition area between two biomes or different patches of the landscape. An ecotone may appear on the ground as a gradual blending of the two communities across a area or it may be a sharp boundary line (e.g., grass land to forest).
ectomycorrhiza	A mutialistic symbiosis between the hyphae of certain basidiomycetes and ascomycetes and the fine roota of certain plants.
effluent	Liquid waste or sewage discharge into a river or the sea (e.g., the bay was contaminated with the effluent from an industrial plant).
euthrophication	Process of degradation in aquatic environments caused by nitrogen and phospurus pollution and characterized by algal blooms and oxygen depletion.
global climate change	Climate characteristics that are changing now and will continue to change in the future, resulting in part from human activity.

Goemycology	The scientific study of the roles of fungi in processes of fundamental importance to geology and the biogeochemical importance of fungi is significant in several key areas. Which include nutrient and element cycling, rock and mineral transformation, bioweathering, mycogenic biomineral formation and interactions of fungi with clay minerals and metals. These processes can occur in aquatic and terrestrial environments.
hydrochemistry	A subdivision of hydrology that deals with the chemical characteristics of the water on and beneath the surface of the Earth. Water in all forms is affected chemically by the materials with which it comes into contact, and it can dissolve many elements in significant quantities. Chemical hydrology is concerned with the processes involved and thus includes study of phenomena such as the transport of salts from land to sea (by erosion of rocks and surface runoff) and from sea to land (by evaporation, cloud formation, and precipitation) and the age and origin of groundwater in desert regions and of ice sheets and glaciers.
hypha (-ae)	Long tube-like elements that make up the body (mycelium) of a fungus; may or may not be separate.
in-situ	In the original position.
interdisciplinary	Of relating to, or involving two or more academic disciplines that are usually considered distinct.
infiltrate	To cause a liquid to permeate by passing through interstices or pores.
keystone species	A species that has a disproportionate impact (relative to its numbers or biomass) on the organization of an ecosystem. Loss of keystone species has far reached consequences for the ecosystem.
lignin	The organic substance that, with cellulose, forms the structural basis of most wood tissues.
mycelia	Fungal network of thread like cells.
mycelium	The body of filamentous fungus, Composed of a network of complexly branch hyphae.

mycofiltration	The use of fungi as a membrane for filtering out microorganisms, pollutants and silt. Habitat infused with mycelium reduce downstream particulate flow, mitigate erosion, filter out bacteria and protozoa, and modulate water flow through the soil (Stamets 2006).
mycoflora	All species of fungi that in habit a given area (=mycota).
mycoforestry	The use of fungi beneficial to trees to aid the regeneration of forests (e.g., the establishment of a new forest on land devastated by repetitive slash-and-burn clear cutting practices).
mycologist	A person whom studies the kingdom of fungi.
mycology	The study of fungi.
mycopesticides	The use of fungi like, Cordyceds to infect and then kill insects like carpenter ants from investaion and deteration of your home.
mycoremediation	Mycoremediation centers on the use of fungal mycelium to degrade pollutants in situ. (e.g. to degrade an oil spill on land by mixing or layering mycelium onto the polluted soil.)
mycorestoration	The use of fungi to repair or restore the weakened immune system of the environments. Whether the habitat is damaged by human activity or natural disaster saprophytic, endophytic, mycorrhizal and in some cases parasitic fungi can aid recovery.
mycorrhizal (-ae)	A symbiotic state wherein mushroom mycelium forms on or in the roots of trees and other plants. Mycorrhizae are either endo or ecto.
niche	The function of position of an organism or population with in a ecological community. The particular area within a habitat occupied by an organism.
Phytoremediation	The use of plants to remove or neutralize contaminants as in polluted soil or water.
Precautionary Principle	Principle stating that it may be better to avoid taking a particular action due or the possibility of causing unexpected harm (e.g. choosing not introduce a non-native species like scotch broom to promote slope stability because it will out compete native plants).

primary producers	An organism such as green plants, alga, or seaweed that obtains its energy directly from the sun via photosynthesis. Also known as an autotrophy or photosynthetic species.
resilience	The ability of an ecosystem to return to its original state following disturbance.
saprophyte	A fungus that lives on dead organic matter. [These fungi] are among the first organisms to rejuvenate the food chain after catastrophe.
Species diversity	all the species on earth, including single-celled bacteria and protisits as well as the species of the multicellular kingdoms (plants, fungi, animals).
species richness	The number of species found in a community. Species richness increase with decrease in elevation, increasing solar radiation increasing precipitation, hot raining low lands have the most species.
succession	The gradual replacement of one group of organisms by another over time following initial disturbance.
sustainability	Characteristics by which a process or state can be maintained at a certain level indefinitely.
sustainable harvest level	Level of wood, plant, or fungi harvesting that can be sustained indefinitely. In forestry this is often calculated as annual allowable cut on per-year basis for any specific region.
sustained yield	Amount of natural resource, such as fungi, that can be harvests without reducing the inventory or production potential.
symbiotic relationship	A close long term biological relationship in which two species are always found living together.
trophic levels	Levels of biological communities representing ways in which energy is captured and moved through the ecosystem by the various types of species. Primary producer; herbivore, secondary consumers are carnivore and detritivore.
watershed	A ridge of high land dividing two areas that are drained by different river systems. The region draining into a river, river system, or other body of water.

white rot	A condition whereby a substrate is rendered light in color from the fungal decomposition of lignin (delignification), leaving cellulose largely intact. Solid blocks of wood can be utilized for testing whether fungus causes white rot or brown rot.

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