Fungal Bioremediation in China

Honors Capstone 2009

Althea Mickiewicz

Advisors:

Yang Qing
Masters Student
College of Environmental Science and Engineering, Peking University

Professor Huang Yi
College of Environmental Science and Engineering, Peking University

Professor Albert Cheh
College of Arts and Science, American University

Introduction

DDT (**D**ichloro-**D**iphenyl-**T**richloroethane) is a persistent organic pollutant, meaning it does not breakdown easily but instead accumulates in the food chain of natural ecosystems. An organochlorine insecticide, DDT was used extensively in the 1950's to increase crop yields. Despite international conventions in the 1970's to limit use of DDT to malaria applications, developing countries especially still struggle with toxic residues. China particularly feels the need to rehabilitate contaminated arable land, 20% of which has been deemed unsuitable for agriculture.

Ectomycorrhizal Fungi (ECMF) are organisms that form a symbiosis with plant roots. Their mycorrizae surround the roots, extending the plant's ability to reach water and nutrients while receiving sugars in return. The ECMF, *Xerocomus chrysenteron* (Xc), exudes ligninolytic enzymes found to degrade DDT. The potential for enhancing this process for bioremediation of persistent toxins and heavy metal contamination is of interest to the Chinese government and the environmental community at large. Through a laboratory study with the College of Environmental Science at Peking University, we investigated the relationship between Xc enzyme activity and DDT. Our findings indicate more research should be applied to this process before undertaking large scale application. Questions remain concerning what happens to DDT degradation products and how best to apply this strategy of bioremediation.

Environmental Context

Persistent organic substances include a variety of pollutants that do not degrade readily. This allows harmful chemicals to cycle through the environment and accumulate in the food chain. One of the most infamous pollutants is dichlorodiphenyltrichloroethane (DDT). Used as an insecticide to increase crop yields, DDT reached its peak use after World War II.

China has continued a heavy reliance on pesticides. Though the most toxic types have been banned, aggregate use continues to rise (Hamburger, 2002). DDT was phased out of agriculture in 1983 but is still allowed for malarial use. Often such a partial band makes illegal pesticides easily available for use on small farms. 70% of China's pesticide use is considered highly toxic by the government.

Another pollutant type of concern is heavy metals. Toxins such as lead, mercury, cadmium leach from landfills containing electronic waste (Choi, 2007). Smelting plants are a significant source of these heavy metals. Mercury and cadmium are particular issues in river contamination. Residues accumulate in fish stocks and continue up the food chain (Brownlow, 2007). These chemicals pose health risks to humans working closely to the material as well as ecological risks to the surrounding ecosystem.

Large-scale animal husbandry contributes to heavy metal pollutants. These include copper, zinc, selenium, cobalt, arsenic, iron, and manganese (Ellis, 2007). Used to increase livestock weight gain and appearance, the heavy metals are excreted back into the soil.

Approximately 20% of China's arable land has been deemed too polluted for agricultural use (Li, 2006). The country's historical focus on high yield production at the cost of ecosystems during the Green Revolution leaves its mark both mentally and physically. Desertification, salinization, erosion, overgrazing, industrial pollution, and pesticide contamination all combine to describe a dire picture of China's arable land today (Hamburger, 2002).

Current Initiatives

Restoring China's arable land to agricultural use is a government priority. Natural attenuation or bioremediation is an attractive alternative to the costly effects of pollution and manual cleaning efforts. China is interested in developing strategies to remove, volatize, degrade, or stabilize contaminants. Practice plots demonstrating phytoremediation of As and Cd have had success (Luo, 2006). Microbial remediation has been used to target persistent organic pollutants like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Detailed examinations of these mechanisms are underway. In some cases, the responsible genes are being sequenced and cloned to reproduce the effect.

Laboratory Investigation

Ectomycorrhizal fungi (ECMF) are organisms that form symbiotic relationships with plant roots in order to exchange nutrients. In exchange for sugars, ECMF increase the surface area of the root system through an extensive mycelial network. One such ECMF, *Xerocomus chysenteron* (Xc), not only helps transfer nutrients to its host plant but also may protect it from harmful toxins in the soil. Also known as *Boletus chysenteron,* or Red Cracking Bolete, Xc is a common fungus to many temperate zones. Its above ground form is a typical mushroom that is consumable by humans. Most significantly, Xc exudes enzymes that have been observed to degrade persistent organic pollutants such as DDT. Combined with a tree species native to China, *Pinus sylvestris*, Xc is an ideal candidate for bioremediation of the country's many contaminated sites. Though the effect of ECM fungi on DDT concentration is documented, the mechanism by which it operates is still unknown. Over a semester spent working with the College of Environmental Science at Peking University, a variety of tests were used to evaluate the processes involved in the ECMF symbiosis.

Methods and Procedures

Azure B

Laccase has been found to degrade DDT naturally in the environment. After 54 days of DDT exposure to laccase, the deteriorating effect is evident using both a 10 parts per million (ppm) and 5ppm DDT starting conditions. The main experiment goal is to identify what enzymes are most critical in this process. Testing for different enzymes one

by one is the current method of determining which are active during the fungal growth. The following procedure sets up an experiment in which lignin peroxidase would be positively identified if it reacts with the Azure B agar solution with the result of a white color change. To sterilize the Petri dishes, all were wrapped up in newspaper in a cylindrical fashion in groups of 4. These were set aside while the agar solution was prepared. A 500mL beaker was washed with soap and tap water.

In an adjacent lab room the ingredients were measured out onto an electric, sliding door scale. 3.0023g glucose was weighed on weighing paper and deposited into a 50mL beaker. 0.03g Yeast extract was weighed on the same paper and added to the 50mL beaker. 0.0299g Azure B was weighed out and added to the 50mL beaker. 5.4448g agar was weighed and put in another 50mL beaker before returning to the original lab room. 300mL purified water was measured into a 500mL graduated cylinder to rinse the 50mL beaker mixture into the 500mL beaker. The 50mL Agar beaker was added to the solution pouring along a stirring rod to add the remaining water. The 500mL beaker was covered with parafilm secured by rubber bands.

3 core borers and 1 set of tweezers were wrapped in newspaper. These wrapped items, along with the wrapped Petri dishes and 500mL beaker solution, were placed in a LD2X-40B Automatic vertical electro thermal pressure steam sterilizer (referred to as oven) and heated at 122 degrees Celsius. This device came to about waist height and required the addition of approximately 2 Liters of water poured over the first lid before securing the second lid and turning on. The oven indicates it is done after an indefinite amount of time by emitting a loud noise.

After about an hour in the pressure steamer, the sterilized dishes and tools were removed and brought into the clean room. Before entering the sterile and cold room, all laboratory assistants put on lab coats and gloves. Inside the room experimenters used ethanol to sterilize their gloves. The prepared agar solution was poured into 11 Petri dishes (one sterilized dish was neglected due to too much agar solution being poured into other dishes). The optimal level of agar was about 1 cm thick. Once the agar had cooled the core borer were sterilized in the flame of a kerosene lamp and used to make 11 removable impressions in fungus colonies in a Petri plate. Tweezers were sterilized by fire and used to transfer the circular fungus samples to the cooled agar plates. Each transferred piece was placed fungus-side-up in the center of the plate. The prepared plates were sealed with parafilm and labeled with the date, fungus type (X_c), and preparer's name. The 11 plates were put in an incubator set at 25 degrees Celsius and were to remain there for 5-10 days, watching for a color change. All metal instruments were sterilized in fire after use.

EMCF Assay Preparation

To set up culture filtration, 31 50mL flasks/beakers were allowed to cool after sterilization in oven. Non-DDT cultures were poured over filter paper after an attached vacuum pump was turned on. The pump used water circulation to create a vacuum, drawing liquids through the filter paper and into the flask. Waiting time is approximately 3 minutes until the liquid all filters through. Filtered liquid was poured into clean beaker, re-labeled with the date, and sealed. Used filter was thrown away and filter holder was

rinsed with distilled water. A total of 26 samples were filtered; 14 without DDT were filtered first to avoid contamination and 12 with DDT were filtered next.

14 samples were tested for LiP enzyme; 7 of the DDT solution and 7 of the non-DDT solution from the same dates. 16 test tubes were prepared, including to blanks with only water. 2.2mL of each beaker sample were pipetted into a respective test tube and labeled each "A" to signify the first testing day. 0.1mL Methyl blue, 0.3mL Sodium tartarate buffer, and 0.1mL H_2O_2 [4.5] were also added to each test tube.

Using a spectrophotometer samples were tested for absorbance after a preparation procedure was performed based on three different assays for the enzyme lignin peroxidase. The Azure B assay included the following preparations:

For a system of 3mL at λ= 651nm

Sodium tartarate buffer (pH 3.0)	1mL (125mM/L)
Azure B	500µL (0.160mM/L)
Culture filtrate	500µL
Hydrogen peroxide solution (H_2O_2)	500µL (2mM/L)

In the blank run, only the 100mL of H_2O_2 was added before insertion into the spectrophotometer. For the non-DDT solutions used, 0.5mL Azure B, 1 mL of culture filtrate and 100mL of H_2O_2 was added. For the DDT solutions 0.5mL Azure B, 1mL culture filtrate, 1mL Sodium tartarate and .5mL H_2O_2 was added.

Veratyl Alcohol assay (VA) included the following preparations:

For a system of 3mL at λ= 310nm

Sodium tartarate buffer (pH 3.0)	0.6mL (250mM/L)

VA	0.6mL (10mM/L)
Hydrogen peroxide solution (H_2O_2)	1.2mL (1mM/L)

Methylene blue (MB) assay included the following preparations:

For a system of 3mL at λ= 664nm

Sodium tartarate buffer (pH 4.0)	0.6mL (0.5mM/L)
MB	0.1mL (1.2mM/L)
Culture filtrate	2.2mL
Hydrogen peroxide solution (H_2O_2)	0.1mL (2.7mM/L)

Three additional enzyme assays involved the following preparations:

1) Laccase

0.1mL fungus culture extract

2.6mL buffer of pH 4.5

CH_3COOH 0.1m/L

CH_3COONa 0.1m/L

0.3mL ABTS at 0.5mM/L

λ= 420nm

2) LiP

0.6mL tartaric acid (250mM of pH 3.0-3.5)

0.6mL VA (10mM)

1.2mL H_2O_2 (1mM)

0.6mL Xc fungus filtrate

λ= 310nm

3) Manganese peroxide (MnP)

0.6mL Malonic acid (250mM)

0.6mL $MnSO_4$ (25mM)

1.5mL H_2O_2 (0.1mM)

0.3mL Xc fungus filtrate

2g Sodium hydroxide

λ= 270nm

Xc Fungus Preparation for DNA Test

Two solutions of 800mL and 200mL solutions were prepared by pipetting 8mL and 2mL, respectively, of the same solution used in previous DDT culture preparations. Glucose was added in .8g and .2g measurements, respectively. 3.6 g of agar was added to the 200mL solution after obtaining the optimal pH 5.5. To obtain the correct volume, 752mL of water was added to the 1000mL flask (for the 800mL solution) and 188mL water was added to the 250mL flask (for the 200mL solution). After sterilization in the pressure oven, the 200mL solution was divided and solidified in 8 test tubes, at a slant to expose more surface area. The 800mL solution was likewise divided into 4 flasks of 250mL each.

Xc fungus cultures that were incubated on Sept. 17th and 24th, 2007, were prepared for a DNA test (PCR) by adding distilled water, agitating the test tubes, and using the resulting microbial solution. Each of the six 250mL flasks was prepared with

5mL of the Xc fungus culture solution while each test tube received 1mL of the same solution. The new culture samples were labeled and stored in an incubator for future use.

DNA Extraction form Xc Fungus for PCR Test

After 7 days, one fourth of the Xc culture flasks showed no growth and only the 4 flasks with the most growth were used. The fungus was isolated using a water-circulation vacuum pump to filter the solution. Using tweezers, the small amounts of fungi remaining on the filter paper were transferred into 4 closed tubes. The tubes were then spun in a centrifuge for 10 minutes. With a mortar and pestle, the spun fungus was ground in combination of liquid nitrogen. The next step would have been to continue using a TakaRa Universial Genomic DNA Extraction Kit (3.0), but the procedure was postponed due to lack of technical understanding/equipment.

Tree Preparation

Pinus tabulaeformis seeds were prepared for a new batch of planting. Last batch in two 10 cm diameter porcelain dishes were found dead and organism infected and disposed of in clear plastic bag. New seeds were brought out in a 7cm by 14 cm clear plastic bin. Seeds were rinsed in tap water, and then strained. 10mL 30% H_2O_2 added to 20mL H_2O to make 10% H_2O_2. The H_2O_2 solution was poured into the plastic bin until the seeds were covered. The sterilized seeds were mixed with a magnetic stirrer for 10-30 minutes. The seeds were then drained and rinsed with tap water. Water temperature was monitored for optimal gestation by adding 60 degree Celsius water to cool the 68

degree Celsius tap water remaining with the seeds. The seed bin was placed in incubator set at 60 degrees Celsius.

Maintaining the trees requires watering each of the approximately 15 tubs with planted seedlings in white soil-substitute. The water used was purified and each tub received 500mL from a beaker. Each tub contained about 6 seedlings planted within the within the last 30 days in about 20 cm of soil-substitute. The plants receive nutrients from an outside source as the soil substitute contains no nutrients.

Pinus tabulaeformis was chosen to be used in this experiment for quick growth. However, the seeds prepared showed no growth after one week. The incubator had at one point been turned up to approximately 67 ºCelsius although the optimal temperature for the seeds' germination is 60 ºC. This may have killed the seeds or stunted their growth and would be a good procedural aspect to be careful about in future experiments.

Results

The spectrophotometer results come from the LiP enzymes reaction in the various assays. Using the H_2O_2 as primary oxidant, evidence of the enzyme's activity is demonstrated by an increase in absorbance readings. The data for four successive 1minute readings was recorded during each assay. Initial data was unusable because of procedural error and successive readings were inconclusive as to the presence of the LiP enzyme. Qualitatively, we investigated the enzyme activity of Xc through blue dye discoloration. The activity of the enzyme process bleaches the blue dye so that a positive test for the enzyme would result in discoloration of the plate. The prepared fungus and Azure B Petri dishes were checked 20 hours after initial incubation and a

slight change was observed in coloration from deep blue to more white or clear. The color change centered around the fungal growth, indicating the activity of an enzyme, lignin peroxidase (LiP), that has been determined to break down DDT and other pollutants. The mechanism of this process is unclear; LiP may use the pollutants as main source of nutrients or degrade them as a byproduct of its metabolic process. However, LiP has been shown to be less effect in degrading higher concentrations of pollutant without another source of nutrients (Burke, 2002).

Quanitatively, we used mass spectrometry to monitor the absorbance of a suspended fungi culture. The presence of each product showed by how much light was able to pass through the liquid sample. Quantitative analysis did not show a significant trend among product abundance over time. However, the presence of Difocol indicates that perhaps enzyme activity is affecting a product of DDT.

Discussion

Challenges to our laboratory study included the time required to grow pine trees sufficiently for inoculations with fungi culture. A semester long period was not enough to repeat experimental procedures to produce a meaningful result.

Also, the risk of bacterial infection threatened fungi cultures and analysis. The Azure B test showed one plate had clear evidence of bleaching. A similar show of enzyme activity did not occur for any other plate. This may have been due to bacterial infection that competes with fungi for nutrients and thereby limits fungal activity (GAO et al., 2004).

Lack of available C-14, used as a stable isotope to trace DDT uptake, limited what we could find about where toxic products go and in what amounts. Before field applications are put into practices, more needs to be known about the bioremedial activity of Xc and its enzymes. C-14 tracing would be a simple method for such analysis.

Ecological Context

Species Interactions

Mycorrhizal fungi act on ecosystems in widely different ways. Direct association with root systems, make supplying nutrients a main role. In effect, mycorrhizal fungi extend the reach of the roots into soil to help the plant take up water and minerals. By limiting the uptake of certain soil matter, mycorrhizae also play a protective role to plant roots.

The two subgroups of mycorrhizae are endomycorrhizas and ectomycorrhizas. Endomycorrhizas, including arbuscular, arbutoid, ericoid, orchid, and monotropoid fungi, typically enter into the host plant and perform functions intracellularly (He et al., 2007). Ectomycorrhizas are more commonly interactions between woody plant and fungi. Forming wide networks between tree roots under the soil, ectomycorrhizas perform mostly extracellular functions. This strategy allows fungi to modify toxins before they enter the host species.

Arbuscular mycorrhizal fungi (AMF) is interesting for its effect on several elements. He et al. classified nutrients into large and small subgroups. Macro-nutrients includes nitrogen, phosphorous, and potassium (He et al., 2007). Micro-nutrients are the heavy metals copper, iron, and zinc. For the organism He et al. studied, the AMF took up these nutrients, helping to transport appropriate amounts to the plant. This phenomenon is so common that mycelia and associated exudates can make up one third of soil's total microbial biomass.

A study of ectomycorrhizal fungi (ECMF) compared the symbiotic organism to white rot fungi (Meharg et al., 2000). White rot, often considered a blight to many woody plant crops, is a successful agent of bioremediation. Containing the tough liginolytic enzyme, laccase to break down woody tissue, white rot may have uses to bioremediate resilient pollutants such as DDT (Hawaii University, 2009). Meharg et al. found that although tree root colonization was slower for ECMF, the fungi worked more extensively than white rot. ECMF proved to be more aggressive in branching out and creating soil networks.

One ECMF species, *Pisolithus tinctorius*, is known for its ability to reclaim poor, contaminated soils. Resistant to drought and tolerant to limited nutrients, *P. tinctorius* can function under high acidity, heavy metal concentration, and temperatures (Volk, 2003). Paired with the fast growing host tree, *Eucalyptus tereticornis,* the fungi accumulates aluminum, cadmium, chromium, iron, lead, and nickel (Ray et al., 2005). These contaminants are typical of industrial residues from mineral mining and resource extraction. Ray et al. used atomic absorption spectrophotometry to show that ECMF tolerance varied within and among species.

The tree species, *Pinus sylvenstris*, can grow in association with several different ECMF. With *Amanita rubescens* inoculation, more tree growth is seen as well as a higher accumulation of Cd(II) (Kozdroj et al., 2007). Furthermore, the same study showed the benefits of fungal association with bacteria. *Pseudomonas putida* combined with *A. rubescen* treatments correlated with more tree growth than seedlings without. Therefore, the study of EMCF should not be confined to a simple, two species analysis.

Mechanisms Involved

Of the many ways fungi interact with host plants, extracellular enzyme activity is among the most promising. Studying several ECMF, Courbet et al. found *Paxillus involutus* increasing the enzyme glutathione in response to cadmium (Courbet et al., 2004). Along with other thiol compounds such as phytochelatins and metallothioneins, the increased production of this enzyme in the presence of cadmium indicates there may well be a positive feedback mechanism that would increase the fungi's ability to chelate heavy metal ions in the plant's cytosol.

Brown rot, similar to white rot, fungi are recognized agents of bioremediation and not just household annoyances. Norway spruce (*Picea abies*), brown rot causes copper and chromium to leach out of contaminated wood tissue (Humar et al., 2003). The oxalic acid excretion of brown rot is seen as the critical step in this remedial process. Grasses as well as trees serve as host organisms to fungal experiments. Sorghum inoculated with *Gigaspora rosea* and *Glomus mosseae* employed glomalin excretions to stabilize toxic deposits of copper, cadmium, lead, and magnesium (Gonzalez-Chavez et al., 2004). Glomalin remains a key focus for scientists interested in exploring its biostabilization properties.

Future Research

Nearing the end of the 11th five year plan, China is approaching a time of reflection on past efforts and strategies going forward. By applying these naturally occurring interactions to contaminated sites could help facilitate hazardous pollution clean-up. But some key questions remain. If we use plants to bioremediate toxic

elements, where does the toxin actually go? What happens when we harvest plants? Suggested solutions include phyto-mining, transfer to metal-deficient soil, biomass burned for energy, and even human health applications (Luo, 2006).

Among the challenges in pursuing large-scale application of fungal bioremediation strategies, even identifying species can be a daunting task. Different fungi species may look alike on same plant so DNA sequencing is necessary to sort taxa out (Davis et al., 2002). Still, finding PCR primers to distinguish between the plethora of mycorrhizal fungi is difficult.

Laboratory conditions, too, appear to be a common problem of obtaining clear results. Bacteria grow faster than fungi and, once cultures are infected, they can overrun ECMF and white-rot colonies (GAO et al., 2004). One solution is preparing a nitrogen-limited medium that is inhospitable to bacterium.

More research needs to be done before these technologies can confidently be exploited for ecological benefit. Some have already called for multidisciplinary study and international cooperation (Luo, 2006). Many researchers would like to see more studies *in situ*. Laboratory studies are not as immediately useful as fieldwork, but more appropriately cautious when so many toxin uptake pathways remain unclear. Some of the most successful laboratory studies have been conducted using liquid culture media (Singh, 2006). This is because conditions for species interaction can be highly monitored and controlled. The same advantages apply to bioreactors, where contaminated soils are treated in concentrated mixtures of remedial agent.

Conclusion

Pollution is no longer simply a local issue, it is increasingly a global issue. The same toxins affecting a village in China find their way by land and sea to communities in North America. These two regions specifically are under a lot of focus for both their role in causing and remediating pollution. A globalized food system and a growing world population make every natural resource more valuable. Where land productivity suffers due to pollution, much is to be gained by developing safe, cost-effective ways to reestablish functioning ecosystems. Fungal bioremediation is a lesser known method of rehabilitating agricultural land. Toxins such as DDT and heavy metals have been reduced either by degradation or sequestration in soils through symbiotic relationships between roots and fungi. The potential for enhancing this process and applying it on a large scale is an attractive policy option for the Chinese government. However, obstacles remain as technical capacity is limited by knowledge of what specific mechanisms can be exploited. More attention, research, and resources dedicated to fungal bioremediation would go a long way into creating efficient solutions for persistently polluted soils.

Works Consulted

Archibald, Federick S., 1992. A New Assay for Lignin-Type Peroxidases Employing the Dye Azure B. *Applied and Environmental Microbiology*. Vol. 58, pp. 3110-3116.

Adriaensen, K., T. Vralstad, J.-P. Noben, J. Vangronsveld, and J. V. Colpaert. 2005. Copper-Adapted Suillus luteus, a Symbiotic Solution for Pines Colonizing Cu Mine Spoils. Applied and Environmental Microbiology. 71:7279-7284.

Bellion, Marc, Mikael Courbot, Christophe Jacob, Frederic Guinet, Damien Blaudez, and Michel Chalot. 2007. Metal induction of a Paxillus involutus metallothionein and its heterologous expression in Hebeloma cylindrosporum. New Phytologist. 174:151-158.

Brownlow, Kaleb and Stephanie Renzi. 2007. "Is Guangdong the Dark Horse in Addressing Ecological and Human Health Threats?" China Environmental Series. Woodrow Wilson International Center for Scholars. 9:43-56.

Burke, R.M., Cairney, J.W., 2002. Laccase and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. *Mycorrhiza*, Springer-Verlag. 12: 105-116.

Burke, R.M., Cairney, J.W., 1998. Do ecto- and ericoid mycorrhizal fungi produc peroxidase activity? *Mycorrhiza*, Springer-Verlag. 8: 61-65.

Cairney, J.W.G. and S.M. Chambers. Ectomycorrhizal Fungi. Springer-Verlaf Berlin Heidelberg: New York (1999).

Choi, Jamie. 2007. "High Tech's Toxic Legacy in China." China Environmental Series. Woodrow Wilson International Center for Scholars. 9:77-81.

Courbot, Mikael, Laurent Diez, Roberta Ruotolo, Michel Chalot, and Pierre Leroy. 2004. Cadmium-Responsive Thiols in the Ectomycorrhizal Fungus Paxillus involutus. Applied and Environmental Microbiology. 70:7413-7417.

Davis, Lawrence C., Sigifredo Castro Diaz, Qizhi Zhang, and Larry E. Erickson. 2002. Benefits of Vegetation for Soils with Organic Contaminants. Critical Reviews in Plant Sciences. 21:457-491.

Damian, Luminita, Didier Fournier, Mathias Winterhalter, and Laurent Paquereau. 2005. Determination of thermodynamic parameters of Xerocomus chrysenteron lectin interactions with N-acetylgalactosamine and Thomsen-Friedenreich antigen by isothermal titration calorimetry. BMC Biochemistry. 6:11.

Donald, D.G.M., T.W. Hu, W-E. Cheng. 1988. A study of the mycorrhizal associations of Paulownia taiwaniana. South African Journal of Plant and Soil. 5:79-83.

Donnelly, Paula K., James A. Entry, and Don L. Crawford. 1993. Degradation of Atrazine and 2,4-Dichlorophenoxyacetic Acid by Mycorrhizal Fungi at Three Nitrogen Concentraions In Vitro. Applied and Environmental Microbiology. 59:2642-2647.

Ellis, Linden J. and Jennifer L. Turner. 2007. "Surf and Turf." China Environmental Series. Woodrow Wilson International Center for Scholars. 9:25-35.

Finlay, Roger D. 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity involving the extraradical mycelium. Journal of Experimental Botany. 59:1115-1126.

Foght, Julia, Trevor April, Kevin Biggar, and Jackie Aislabie. 2001. Bioremediation of DDT-Contaminated Soils: A Review. Bioremediation Journal. 5:225-246.

GAO, Dawen, Xianghua WEN, and Yi QIAN. 2004. Decolorization of reactive brilliant red K-2BP with white-rot fungi under non-sterile conditions. Chinese Science Bulletin. 49:981-982.

Gaur, Atimanav and Alok Adholeya. 2004. Prospects of Arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science. 86:528-534.

Gramss, G., Th. Gunther, and W. Fritsche. 1998. Spot tests for oxidative enzymes in ectomycorrhizal, wood-, and litter decaying fungi. Mycological Research. 102:67-72.

Hamburger, Jessica. 2002. "Pesticides in China." China Environment Series. Woodrow Wilson International Center for Scholars. 5: 29-44.

Hawaii University. 2009. "White-rot Fungus." Bioremediation technologies. http://www.hawaii.edu/abrp/Technologies/fungus.html (April, 2009).

HUANG, Yi, Ting LI, and Zhi-ji HUANG. 2007. Ectomucorrhial fungi induced change of heavy metal speciation in the rhizosphere of Pinus tabulaeformis seedlings. Department of Environmental Sciences.

Humar, M., M. Bokan, S.A. Amartey, M. Sentjurc, P. Kalan, and F. Pohleven. 2003. Fungal bioremediation of copper, chromium and boron treated wood as studied by electron paramagnetic resonance. International Biodeterioration & Biodegradation. 53:25-32.

Kretzer, Annette M., Susie Dunham, Randy Molinas and Joseph W. Spatafor. 2005. Patterns of vegetative growth and gene flow in vinicolor and R. vesiculosus (Boletales, Basidiomycota). Molecular Ecology. 14:2259-2268.

Li, Zijun. 2006. "Soil Quality Deteriorating in China, Threatening Puclic Health and Ecosystems." Worldwatch Institute. http://www.worldwatch.org/node/4419 (March, 2009).

LUO, Yongming and Yongguan ZHU. 2006. "Bioremediation of Polluted environments: Focus on soil bioremediation in China." Kavli Frontier of Science Symposium Series. National Academy of Sciences. http://www.nasonline.org/site/DocServer/CAFOS06-LuoYongming.pdf?docID=20809 (April, 2009).

Pointing, S. B. 2001. Feasibility of bioremediation by white-rot fungi. Applied Microbiology andl Biotechnology. 57:20-33.

Ray, Prasun, U. Gangi Reddy, Frederic Lapeyrie, and Alok Adholeya. 2005. Effect of Coal Ash on Growth and Metal Uptake by Some Selected Ectomycorrhizal Fungi In Vitro. International Journal of Phytoremediation. &:199-216.

Singh, Harbhajan. Mycoremediation: Fungal Bioremediation. John Wiley & Sons, Inc. Hoboken, New Jersey: 2006.

Volk, Tom. 2003. "Pisolithus tinctorius." University of Wisconsin Madison. http://botit.botany.wisc.edu/toms_fungi/jun2003.html (February, 2009).

Wu, Yucheng, Ying Teng, Zhengao Li, Xuewei Liao, Yongming Luo. 2007. Potential role of polycyclic aromatic hydrocarbons (PAHs) oxidation by fungal laccase in the remediation of an aged contaminated soil. Soil Biology & Biochemistry. 40: 789-796.