To the Graduate Council:
I am submitting herewith a dissertation written by Sharon Rose Jean-Philippe entitled "The Effects of Mercury Contamination on Tree, Fungal, and Soil Composition along East Fork Poplar Creek, Anderson and Roane Counties, Tennessee." I have examined the final electronic copy of this dissertation for form and content and recommend that it be in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Natural Resources.

Jennifer A. Franklin, Major Professor

We have read this dissertation
And recommend its acceptance:

Nicole Labbé

Karen Hughes

David Buckley

Accepted for the Council:

Carolyn Hodges
Vice Provost and Dean of the Graduate School

The Effects of Mercury Contamination on Tree, Fungal, and Soil Composition along East Fork Poplar Creek, Anderson and Roane Counties, Tennessee

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Sharon Rose Jean-Philippe
May 2010

ACKNOWLEDGEMENTS

First of all I would like to thank my major professor Dr. Jennifer Franklin for her support that she provided throughout this research project. I was grateful for the financial support that she found to allow me to come and study under her tutelage. I also especially thank my committee: Dr. Nicole Labbè, Dr. Karen Hughes, and Dr. David Buckley. Thank you for your much needed and appreciated input into my project.

Thank you to Nicolas Andrè, who without his guidance in sample preparation and spectroscopy logistics I would not have left home base. Thanks to Ann Reed for statistical assistance, Timothy Prather for GIS assistance and Dr. Amy Johnson's lab technicians Cassi Jones and Ryan Blair for guidance in soil experimental methods and analysis. I would also like to thank Brien Ostby, Stuart Wilson, Douglas Mitchell, Stephanie Jackel, Jeremie Damay, Steven Grayson, Chris Miller, Jordan Marshall, and John Johnson for their help in field and laboratory work during my project.

Thank you to the Black Graduate Fellowship, J. Wallace Katie Dean Graduate Fellowship, Southern Regional Educational Board (SREB), and Agricultural Minority Award for assistance and most of all financial support during my graduate tenure. Also without The U.S. Department of Energy (DOE) permission to access my study area, this research project would not have been possible.

Lastly, but certainly not least, I would like to thank my father David Leon Sparks who is no longer with us and mother, Juanita Sparks for all their wisdom, love, and financial support; this also includes my nine brothers and three sisters. Finally, I can't

forget my husband Smith Jean-Philippe and son Sidney David Jean-Philippe for without them, I wouldn’t be sleep deprived!

ABSTRACT

The Oak Ridge Reservation established under The Atomic Energy Commission was the site for uranium enrichment and the construction of the atomic bomb during the early 1950's and 60's. Unfortunately, large quantities of "heavy metals" such as mercury, uranium, technetium, plutonium and fission products that were produced were dumped into unlined landfills, settling ponds and surface streams. One such creek affected was East Fork Poplar Creek, whose head water begins at the Y-12 Facilities located on the Oak Ridge Reservation, and was once used as an industrial drainage ditch for runoff, which included mercury and other heavy metals.

The release of mercury, in particular, into East Fork Poplar Creek was probably lethal to established seed banks, vegetation, and soil microbial and fungal communities. The soil microbial communities play an important role in ecological processes, and the fungal communities are important, in particular, due to the mutualistic associations shared with more than 85% of plant species. This study evaluating the long term effects of mercury on plant and fungal presence and abundance indicated that soil mercury concentration was not significantly correlated with these factors. In order to better understand the effects of mercury compounds on plant and fungal interaction, a greenhouse study was conducted. Survival of seedlings in mercury-contaminated media was more dependent on mercury compound applied than on the presence of fungal inoculates tested. The ability of four tree species to germinate in different mercury compounds was also investigated. The germination of seedlings in mercury solution was dependent on tree species, mercury compound and concentration.

The detection of mercury in environmental samples was based on conventional methods such as cold vapor atomic absorption spectroscopy (CVAAS), and inductively coupled plasma emission mass spectrometer (ICP-MS). Analysis of mercury and other metals by non-destructive techniques such as infrared spectroscopy, specifically near-infrared (NIR) and mid-infrared (MIR) spectroscopy was investigated. Quantitative analysis of plant foliar tissue exposed to mercury was investigated by NIR, and mercury-contaminated soil was investigated by MIR. Due to mercury's volatility, the ability to differentiate NIR spectra of control versus treated seedlings could not be confirmed through statistical analysis, however MIR spectra obtained from mercury-contaminated soil was used to develop significant calibration models for mercury and several other metals correlated to mercury.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PART I
INTRODUCTION

Introduction

Overview of pollutants in the environment

Primary polluting agents in the environment are anthropogenic in nature. Coal combustion in some rural areas in China has been shown to produce fluoride pollution (Ando et al., 2001). These airborne fluorides contaminate expose food products and disrupt the respiratory tract of humans. In Pakistan, incinerated medical waste products generate a variety of pollutants, including cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb). These metals are released as fly ash and toxic metal in the incinerated ash (Sabiha-Javied and Khalid, 2008). Researchers in the Netherlands analyzed how pollution associated with construction and use of roads affects soil, water and air quality (Van Bohemen and Janssen Van De Laak, 2003). Vehicle combustion, degradation of road surfaces and continual application of road maintenance chemicals disseminate pollutants into the environment (Van Bohemen and Janssen Van De Laak, 2003). These examples illustrate how processes that are beneficial to human well-being are simultaneously detrimental to the environment.

Mercury in the environment

Mercury (Hg) has been used for its unique chemical and physical properties, appearing in household products and commercial applications (Han et al., 2006). At standard temperature and pressure, mercury is a liquid metal (Boening, 2000). Mercury forms salts in two ionic states, mercurous salts (Hg^{+}), and mercuric salts (Hg^{2+}) which are the more common of the two found in the environment (Boening, 2000). Mercury can form organometallic compounds used in industry and agriculture. Elemental Hg, which is

strongly toxic, can form vapors that are only slightly soluble in water and are easily transported in the atmosphere (Boening, 2000). The most common form of Hg is insoluble mercuric sulfide (cinnabar), which is non-toxic (Boening, 2000). Mercury in the environment comes from natural and anthropogenic sources. Natural atmospheric circulation or emissions of elemental Hg^o in the form of particulate matter and aerosols can originate from outgassing of the earth's mantel/crustal material, evaporation from surficial soils, water bodies, vegetation surfaces, wild fires, volcanoes, and geothermal surfaces (Boening, 2000; Schroeder and Munthe, 1998). Anthropogenic emissions can originate from methylation of inorganic mercury through burning of fossil fuels, smelting of lead, copper and zinc ores, and manufacturing operations which all contribute to mercury accumulation in the environment (Boening, 2000; Nriagu and Pacyna, 1988; Revis, 1989; Schroeder and Munthe, 1998). It has been estimated that the annual anthropogenic input of Hg to the environment is as high as 6×10^6 kg/yr (Nriagu and Pacyna, 1988). In addition to anthropogenic sources, natural sources bring the total mercury released to the atmosphere to about 741×10^6 kg.

Considerable amounts of mercury can be added to soils from fertilizers, lime and manures due to agricultural processes (Andersson, 1979). One of the most important sources of mercury in agricultural soils has been from organic mercurials used as seed-coat dressing for the prevention of fungal disease in seeds, mercury sulfide used as a root dip, and phenyl mercuric acetate (PMA) for treatment of apple crab (Frank et al., 1976). The accumulation of mercury in water-ways may come from atmospheric deposition,

effluents of chemical factories, and groundwater contaminated with leachates from agricultural processes, slag dump and landfill waste areas.

Mercury effects on vegetation

Within forest communities, heavy metal pollution has been shown to influence primary processes in plants, fungi, algae and bacteria (Beauford et al., 1977; Fargašová, 1994; Godbold, 1991). Mercury pollution is of particular importance due to its deleterious effects on aquatic plants and animals (Burger et al., 2005; Campbell et al., 2005; Theodorakis et al., 2000), soil microbial, plant and fungal communities (Godbold and Huttermann, 1986; Munthe and Iverfedlt, 1995; Tsai and Olson, 1990). Greger et al., (2005) investigated the accumulation, translocation and release of Hg from six different plant species: garden pea (*Pisum sativum*), spring wheat (*Triticum aestivum*), sugar beet (*Beta vulgaris*), oil-seed rape (*Brassica napus*), white clover (*Trifolium repens*), and willow (*Salix viminalis*). Mercury accumulation occurred in both the roots and shoots of all plant species investigated. There was no gaseous conversion and release of Hg by the foliage sampled, although this has been recorded in other studies (Kozuchowhi and Johnson, 1978). Fargašová (1994) investigated the acute effects of five heavy metals (Pb, Cd, Hg, As, and Cr) on seed germination and root growth of mustard seed (*Sinapis alba*). Metal addition had minimal toxic effect on mustard seed germination, but root growth response to heavy metal exposure reduced root nutrient uptake. Millhollen et al., (2006) investigated atmospheric and soil Hg exposure for three woody species, *Juniperus scopulorum* (Rocky Mountain juniper), *Pinus ponderosa* (ponderosa pine), and *Robinia pseudoacacia* (black locust), and found that foliar Hg levels from these trees were

influenced by atmospheric Hg rather than soil Hg exposure. Godbold and Hutterman (1986) observed reduced root elongation in spruce seedlings exposed to Pb and Hg compounds with methylmercury being intrinsically more toxic. Mishra and Choudhuri (1998) also observed reduction in root and shoot elongation and decreased germination in two rice cultivars exposed to Pb and Hg and noted that Hg was more phytotoxic than Pb in both the root and shoot. Mercury absorption by plant roots is low (Lodenius, 1994; Gilmour and Miller, 1973; Lindberg et al., 1979), with restricted transport of Hg observed in the phloem.

Movement of metals within plant roots

Plants require a relatively small number of elements for their growth and survival (Mishra and Dubey, 2006). Soils can, however, contain non essential elements within the soil solution that despite the selectivity of root cell membranes, still may be taken up and detected in plant tissues in trace amounts. Uptake of solutes takes place through the epidermis of the roots. Since mercury is not required for plant growth, there probably is not a specialized transporter for Hg. Therefore Hg accumulation within plant components is through root cells, via transporters for essential cations (Kim et al., 2002). Research suggests the major pathway that ions follow is from the epidermis to the endodermis of the root following a symplastic entry, meaning from cell to cell via plasmodesmata (Clarkson, 1993). Uptake of metals by plant roots depends on the ionic potential of the metal of interest, soil organic matter content, cation exchange capacity (CEC), and pH. Metals can be absorbed actively or passively, accumulating with macronutrient cations and/or competing for uptake at the plants root tip. Binding of metal ions to root cell walls

occurs via high affinity binding sites and plasma membrane localized transport systems. Uptake of metal ions occurs through secondary transporters such as channel proteins and/ or H^+-coupled carrier proteins (Mishra and Dubey, 2006). Secondary transporters facilitate cation uptake by altering the electrical potential of the plasma membrane, which is negative on the inside of the membrane (Hirsch et al., 1998). There are several types of metal transporter and more than one transport system can exist for one metal (Williams et al., 2000). The zinc-iron permease (ZIP) proteins are a family of metal ion transporters that are found in plants, protozoa, fungi, invertebrates and vertebrates involved in metal ion accumulation and homeostasis (Grotz et al., 1998) These proteins have been implicated in the uptake of Zn and Cd in *Thlaspi* species (Pence et al., 2000). In *Arabidopsis thaliana,* the metal ion transporter IRT1, an iron transporter, has been shown to transport cadmium within the roots (Ernst et al., 2008). The uptake of Pd^{2+} and Cd^{2+} via Ca^{2+}, and Mg^{2+} transporters have been observed in rice roots, but inhibition of these metals was also observed (Kim et al., 2002). Metal transport into plant roots can also be inhibited or enhanced by mycorrhizal fungal association.

Plant and fungal interactions

The soil microbial community plays an important role in energy flow, nutrient cycling, soil structure stability, and decomposition processes. Fungi are important to the community adding diversity, uniqueness and antiquity, as well as providing symbiotic association within the terrestrial environment. Through these symbiotic associations several beneficial mutualisms evolved, endomycorrhizal and ectomycorrhizal, ericoid and orchid mycorrhizal associations. Endomycorrhizae are found to be associated with about

90% of plant families encompassing grasses, forbs, and some woody species, while ectomycorrhizae are found about 10% of plant families, with a vast majority of these being woody species.

Roots colonized by fungi can have hyphal networks that extend several centimeters into the soil, exploiting a large volume of soil more efficiently. Mycorrhizal fungi greatly enhance nutrient uptake in plants. Mycorrhizae are especially important in the absorption and transfer of phosphorus, but increased absorption of zinc, manganese, and copper has also been demonstrated (Raven, 2005).

Fungal cell wall components have been shown to alter movement of solutes into plant roots. Components such as chitin, cellulose, and cellulose derivatives have been found to bind heavy metals (Galun et al., 1983). The fungal sheath can act as a barrier preventing heavy metal transport to the root cortex of its host plant. Some fungi may exhibit a hydrophobic fungal apoplast, restricting apoplastic transport of water and ions (Unestam, 1991). Chelation of heavy metals by fungi has been linked to metal tolerance (Gadd, 1993). Several authors point out that mycorrhizal fungi exude organic acids (e.g. oxalic acid) and produce slime capable of binding metals within the fungal components (Lapeyrie et al., 1987; Denny and Ridge, 1995). Several fungal structures, or chemical substances released by fungi have been implicated in protecting plants from heavy metals in contaminated areas. The ability to restricted heavy metals entry into plant roots was shown to be due to the abundance of extramatrical mycelium (Galli et al., 1994).

Enhanced mycorrhizal infection has been shown to be involved in heavy metal uptake and translocation of Cu, Zn, and Co from low concentration soil solutions

(Pacovsky, 1986; Rogers and Williams, 1986). However, Killham and Firestone (1983) have shown in bunchgrass (*Erhanta calycina*) inoculated with the arbuscular mycorrhizal fungus (AMF) *Glomus fasciculatum,* that growth of plant roots and shoots was reduced by increased uptake of Cu, Ni, Pb and Zn. Pine seedlings infected with the ectomycorrhizae fungus *Telephora terrestris* had increased Zn concentrations in needles when exposed to high levels of Zn pollution (Colpaert and Van Assche, 1992). On the other hand, mycorrhizal fungi have been shown to protect plants from heavy metals. Seedlings of *Pinus sylvestris* infected with the ectomycorrhizae fungus *Suillus bovines* had reduced Zn concentration in needles when exposed to high levels of Zn pollution (Colpaert and Van Assche, 1992). Pine seedlings infected with the ectomycorrhizae fungus *Paxillus involutus*, a species isolated from a heavy metal contaminated sites, were used to test the tolerance of those seedlings to increased cadmium (> 15 µM) stress. Non-mycorrhizal pine seedlings exhibited a 35% diminished biomass as compared to seedlings with mycorrhizae, which exhibited no biomass reduction (Schützendübel and Polle, 2002).

Detection of mercury in environmental samples

Mercury concentrations have been determined in numerous environmental samples, including air, water, soil and sediments, fish and shellfish, foods, pharmaceuticals, pesticides, and plant material. The analysis of mercury in environmental samples is complicated by different organic and inorganic forms of mercury species that may be present. Sample preparation varies with the complexity of the sample, but more complex samples require decomposition, following reduction of mercury to its elemental

form. Mercury is relatively volatile, and can be easily lost during sample preparation (Wilken and Hintelmann, 1992). Regardless of these complications, several methods have been developed to determine trace amounts of mercury in environmental samples. To monitor mercury and suspended particulates of mercury in the air, techniques such as cold vapor atomic absorption spectrometry (CVAAS), cold vapor atomic fluorescence spectrometer, atomic fluorescence (AFS) and atomic absorption spectrometry (AAS) are used. To analyze mercury species (inorganic mercury, dimethyl mercury, diethyl mercury, and methyl mercury chloride) in air samples, combing AAS and AFS with gas chromatography (GC) could be used. Numerous methods, including CVAAS, inductively coupled plasma (ICP) mass spectrometer (MS), ICP atomic emission spectrometry (AES), microwave-induced plasma (MIP) AES, neutron activation analysis (NAA), GC/AFS, high performance liquid chromatography (HPLC)/ UV, have been used to determine mercury concentration in aqueous samples. Methods used to detect total mercury in soil samples include CVAAS, x-ray fluorescence (XRF), ICP-MS, and GC / atomic emission dilution (AED). Finally, total mercury determination in plant material includes; CVAAS, and isotope dilution spark source mass spectroscopy (IDSSMS).

Cold Vapor atomic absorption spectrometry

CVAAS is the most commonly used technique for the determination of total mercury concentration in soils, sediments and plant material. The method is based on the chemical reduction of mercuric ions to elemental mercury, typically using tin chloride ($SnCl_2$) or sodium borohydride ($NaBH_4$). The elemental mercury is then swept out of solution with a carrier gas into a long path absorption tube where the absorption at 253.7

nm is measured (Oda and Ingle, 1981). The detection limit for total mercury is around 0.1 mg/L (Becket et. al., 1990).

With the exception of XRF, all previously mentioned methods for soil and plant analysis require digestion or dilution of sample materials with harsh chemicals. Analysis of mercury and other metals through non-destructive infrared techniques such as near-infrared and mid-infrared spectroscopy have not been developed.

Infrared spectroscopy

A compound's infrared spectrum is a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds contained within a molecule (Coates, 2000). Infrared spectroscopy can be used to identify all types of organic and many inorganic compounds in the form of solids, liquids, and gases, with little to no sample preparation. Importantly, the speciation of mercury, methylmercury, ethylmercury and phenylmercury have been determined by Fourier transform infrared spectroscopy (FT-IR) in bacterial cell (Feo and Aller 2000).

The infrared region is divided into of three regions: the near-, mid- and far-infrared. The near-infrared (NIR) region extends from the upper-wavelength end of the visible region at 780 – 2500 nm (12,800 – 4,000 cm^{-1}). Near-infrared spectroscopy is used for quantitative determination of a large number of species, such as water, proteins, fats in food, soil metal content and/or properties, and low-molecular weight hydrocarbons. Spectra obtained from the near-infrared region are overtones of stretching and vibrational bands between C-H, N-H, and O-H (Skoog et al., 1998).

The mid-infrared (MIR) region ranges from 4000 cm^{-1} to 600 cm^{-1}. The mid-infrared region can be used to obtain reflectance spectra from a variety of opaque samples (fabrics, powders, polymers, etc.,) with minimum sample preparation by using an Attenuated Total Reflectance (ATR) accessory. In short, when a beam of radiation passes from a more dense to a less dense medium, reflection occurs. There is penetration of that radiant beam (evanescent wave) into the less dense medium which can vary from a wavelength to several wavelengths. The sample absorbing evanescent radiation causes attenuation of the beam to occur at wavelengths of absorption bands (Skoog et al., 1998).

Statement of Problem

Mercury contamination world-wide has been well documented. In Minamata Bay, Japan, methylmercury discharged from a chemical plant exposed locals to contaminated fish and agricultural vegetation (Harada, 1995). Grain seed exposed to methyl and ethyl Hg compounds were distributed to Iraq, Pakistan, and Guatemala, resulting in dispersal of contaminated food (Bakir et al., 1973). In Oak Ridge, Tennessee, sediments contaminated with Hg discharged from the National Laboratory Y-12 Plant site were used to fill sewer belt lines and used as residential planting material throughout the city (Bashor and Turri, 1986).

In the 1950's and 1960's, a large amount (~ 1,080 metric tons) of mercury compounds was discharged into East Fork Poplar Creek (EFPC) from the United States. Department of Energy's Oak Ridge Tennessee Site (Han et al., 2006; Bashor & Turri, 1986; Revis et al., 1989) (Figure 2.1 in Chapter 2). East Fork Poplar Creek was once used as an industrial drainage ditch for the Y-12 Plant (www.ananuclear.org). Levels of total

mercury in the floodplain soils along this creek ranged from 0.5 to 3000 mg kg^{-1} (Revis et al., 1989). Due to the mercury contamination along EFPC in Oak Ridge, Bashor and Turri (1986) developed an equation to determine the allowable soil Hg concentration. The "crude estimate" they calculated for soil mercury concentration was 12 μg g^{-1}. In calculating this estimate they noted that increased methylation in the soil solution would discount the current estimate. In soil across the United States, total mercury concentration ranges from 0 to 0.2 mg kg^{-1} (Trost and Bisque, 1970). In addition to mercury discharged into unlined landfills, ponds and streams, other significant contaminates such as strontium, tritium, uranium, technetium and plutonium have been released. East Fork Poplar Creek travels through the city of Oak Ridge and feeds into the Clinch River. Mercury has been detected above background levels > 12 μg g^{-1} in the sediments of the Clinch River and in the Tennessee River at Chattanooga, which is 118 miles from Oak Ridge (USEPA, 1989).

Almost sixty years after the discharge of mercury into EFPC, the landscape has changed significantly due to commercial and residential development. Various remediation efforts such as source collection, elimination of untreated discharges, relining of sanitary and storm sewers and bank stabilization have been employed in the Oak Ridge watersheds (Loar, 2004). In the early 1990's Science Applications International Corporation (SAIC) ran transects along EFPC to evaluate soil total Hg levels. The total mercury level ranged from 0 to 200 mg kg^{-1} (SAIC, 1994) with some areas within the floodplain region having greater than 700 mg kg^{-1}.

The effects of mercurials on biochemical and genetic processes in agricultural vegetation have been studied extensively (Bernier et al., 1992; Magos and Clarkson, 1972; De Flora et al., 1994; Fiskesjo, 1969; Krupa and Baszynski, 1995; MacFarlane, 1956). These include disruption of light and dark reactions in photosynthesis (Krupa and Baszynski, 1995), induction of c-mitosis (mitosis that takes place after treatment with colchicin) through disturbance of the spindle activity and somatic mutation of the shot apexes in *Allium* (Fiskesjo, 1969), and binding of mercuric cations to sulphydryl (-SH) groups, disrupting the formation of disulphide bridges for proper protein function (Clarkson, 1972). The forest, soil, and fungal communities established along EFPC were initially exposed to an acute toxic dose of mercury compound(s) for roughly ten to fifteen years (1950-1965). This acute toxic exposure was probably lethal to established seed banks, certain tree species, grasses, terrestrial and aquatic animals, and microbial communities, and caused leaching of important elements (nutrients) from the soil solution and solid phase components. Remediation efforts (1989-1994) brought about additional discharge, which again could be lethal or sub-lethal to the organisms (Alderdice, 1967). What is the general health of a forest community following acute and chronic mercury exposure? The potential mechanisms of amelioration of metal exposure for higher plants and mycorrhizal fungi may include excretion of chelating substances, extracellular sequestration, reduced or modified uptake at the plasmalemma or intracellular detoxification (Jentschke and Godbold, 2000). Also, the soil composition, specifically soil organic matter content, pH, and cation exchange capacity (CEC), can affect the availability of metals present in the soil solution.

Research Objectives

1) Investigate several potential consequences of mercury exposure effects on forested ecosystems. A field study was conducted along EFPC to (1) investigate tree species diversity, (2) identify and quantify mycorrhizal presence, and (3) determine the physiochemical composition of the soil. A lab study was conducted to determine the ability of four tree species, American sycamore (*Platanus occidentalis*), red maple (*Acer rubrum*), shortleaf pine (*Pinus echinata*), and loblolly pine (*Pinus taeda*) seeds to germinate in different mercuric (mercuric nitrate ($Hg(NO_3)_2$ and methylmercury chloride (CH_3HgCl)) solutions, and to determine lethal and non-lethal concentrations for each species tested. American sycamore, red maple and pines are all present within the floodplains of EFPC. *Hypotheses*- (1) Tree diversity decreases as total Hg concentration increases in established vegetation plots. (2) Mycorrhizal abundance and presence decrease as total Hg concentration increases in established vegetation plots. (3) Germination rate of sycamore, red maple and pine seeds decreases as mercuric concentration increases. This objective will be addressed in Part II.
2) Determine whether the mutualistic association shared between American sycamore (*Platanus occidentalis*) and endomycorrhizae enhances tolerance to mercury exposure. A greenhouse study was conducted to (1) quantify mycorrhizal presence post-mercury exposure (2) document sycamore seedling response with and without mycorrrhizae to mercury exposure (3) use near infrared spectroscopy to monitor chemical changes in sycamore foliage due to mercury exposure, and (4) determine mercury distribution in plant tissue by cold vapor atomic absorption spectroscopy

(CVAAS). *Hypotheses*- (1) Seedlings inoculated with mycorrhizae from a contaminated site are more tolerant and survive longer when exposed to mercury contaminated (soil) than non-mycorrhizal seedlings. (2) Mercuric compounds localize more within the roots of sycamore seedlings. This objective will be addressed in Part III.

3) Compare conventional cold vapor atomic absorption spectroscopy (CVAAS) and inductively coupled plasma (ICP) methods for detection of total mercury concentration and multi-element analysis of soil obtained from East Fork Poplar Creek to analysis using mid-infrared spectroscopy. This objective will be addressed in Part IV.

References

Alderdice, D. F. (1967). The detection and measurement of water pollution: Biological Assay. Canadian Fisheries Report No. 9: 33-39, Canadian Fisheries, Ottawa.

Andersson, A. (1979). Chapter 4. The biogeochemistry of mercury in the environment. J. O. Nriagu. Amsterdam, Elsevier/North-Holland Biomedical Press**:** 79-112.

Ando, M., Tadano, M., Yamamoto, S., Tamura, K., Asanuma, S., Watanabe, T., Kondo, T., Sakurai, S., Ji, R., Liang, C., Chen, X., Hong, Z., Cao, S. (2001). "Health effects of fluoride pollution caused by coal burning." The Science of the Total Environment **271**: 107-116.

Bakir, F., Damluji, S. F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N. Y., Tikriti, S., Dhahir, H. I., Clarkson, T. W., Smith, J. C., Doherty, R. A. (1973). "Methylmercury Poisoning in Iraq." Science **181**: 230-241.

Bashor, B., Turri, P. (1986). "A method for determining an allowable concentration of mercury in soil." Archives of Environmental Contamination Toxicology **15**: 435-438.

Beauford, W., Barber, J., Barrington, A. R. (1977). "Uptake and distribution of mercury within higher plants." Physiologia Plantarum **39**: 261-265.

Bernier, M., Carpentier, R., Murtas, N. (1992). Reversal of mercury inhibition in photosystem II by chloride. Proceedings of the IXth International Congress on Photosynthesis, Nagoya, Japan.

Boening, D. W. (2000). "Ecological effects, transports, and fate of mercury: a general review." Chemosphere **40**: 1335-1351.

Burger, J., Campbell, K., Campbell, T., Shukla, T., Dixon, C., Gochfeld, M. (2005). "Use of central stonerollers (Cyprinidae: *Campostoma anomalum*) from Tennessee as a bioindicator of metal contamination " Environmental Monitoring and Assessment **110**: 171-184.

Campbell, K., Campbell, T., Burger, J. (2005). "Heavy metal concentration in Northern Water snakes (*Nerodia sipedon*) from East Fork Poplar Creek and the Little River, East Tennessee, USA." Archives of Environmental Contamination and Toxicology **49**: 239-248.

Clarkson, D. T. (1993). "Roots and the delivery of solutes to the xylem." Philosophical Transactions: Biological Sciences **341**: 5-17.

Coates, J. (2000). Interpretation of infrared spectra, a practical approach. Encyclopedia of

Analytical Chemistry. R. A. Meyers. Chichester, UK, John Wiley and Sons Ltd.**:** 10815-10837.

Colpaert, J. V., Van Assche, J. A. (1992). "The effects of cadmium and the cadmium-zinc interaction on the axenic growth of ectomycorrhizal fungi." Plant and Soil **145**: 237-243.

De Flora, S., Bennicelli, C., Bagnasco, M. (1994). "Genotoxicity of mercury compounds. A review." Mutation Resources **317**: 57-59.

Denny, H. J., Ridge, I. (1995). "Fungal slime and its role in the mycorrhizal amelioration of zinc toxicity to higher plants." The New Phytologist **130**: 251-257.

Ernst, W., Krauss, G-J, Verkleij, J., Wesenberg, D. (2008). "Interaction of heavy metals with sulfur metabolism in angiosperm from an ecological point of view." Plant, Cell and Environment **31**: 123-143.

Fargašová, A. (1994). "Effect of Pb, Cd, Hg, As, and Cr on germination and root growth of *Sinapis alba* seeds." Bulletin of Environment Contamination and Toxicology **52**: 452-456.

Fiskesjo, G. (1969). "Some results from *Allium* test with organic mercury halogenides." Hereditas **62**: 314-322.

Frank, R., Ishida, K., Suda, P. (1976). "Metals in agricultural soils of Ontario." Canadian Journal of Soil Science **56**: 181-196.

Gadd, G. M. (1993). "Interaction of fungi with toxic metals." The New Phytologist **124**: 25-60.

Galli, U., Schuepp, H., Brunold, C., (1994). "Heavy metal binding by mycorrhizal fungi." Physiologia Plantarum **92**: 364-368.

Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. M., Siegel, B. Z. (1983). "Removal of uranium (VI) from solution by fungal biomass and fungal wall-related biopolymers." Science **219**: 285-286.

Gilmour, J. T., Miller, M. S. (1973). "Fate of a mercuric-mercurous chloride fungicide added to turfgrass." Journal of Environmental Quality **2**(1): 145-148.

Godbold, D. L., Huttermann, A. (1986). "The uptake and toxicity of mercury and lead to spruce (*Picea Abies Karst.*) seedlings." Water, Air, and Soil Pollution **31**: 509-515.

Godbold, D. L. (1991). "Mercury-induced root damage in spruce seedling." Water, Air, and Soil Pollution **56**: 823-831.

Greger, M., Wang, Y., Neuschutz, C. (2005). "Absence of Hg transpiration by shoot after Hg uptake by roots of six terrestrial plant species." Environmental Pollution **134**: 201-208.

Grotz, N., Fox, T., Connolly, E., Park, W., Guerinot, M., Eide, D. (1998). "Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency." Proceedings of the National Academy of Science of the United States of America **95**: 7220-7224.

Han, F. X., Su, Y. M, David, L., Waggoner, Charles A., Plodinec, M. J. (2006). "Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA." The Science of the Total Environment **368**: 753-768.

Harada, M. (1995). "Minamata Disease: Methylmercury poisoning in Japan caused by environmental pollution." Critical Reviews in Toxicology **25**: 1-24.

Hirsch, R., Lewis, B., Spalding, E., Sussman, M. (1998). "A role for the AKT1 potassium channel in plant nutrition." Science (280): 918-921.

Jentschke, G., Godbold, D. L. (2000). "Metal toxicity and ectomycorrhizas." Physiologia Plantarum **109**: 107-116.

Killham, K., Firestone, M.K. (1983). "Vesicular-arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions." Plant and Soil **72**: 39-48.

Kim, Y., Yang, Y., Lee, Y. (2002). "Pd and Cd uptake in rice roots." Physiologia Plantarum **116**: 368-372.

Kozuchowki, J., Johnson, D. L. (1978). "Gaseous emissions of mercury form an aquatic vascular plant." Nature **274**: 468-469.

Krupa, Z., Baszynski, T. (1995). "Some aspects of heavy metals toxicity towards photosynthetic apparatus. Direct and indirect effects on light and dark reactions." Acta Physiologiae Plantarum **17**: 177-190.

Lapeyrie, F., Chilvers, G. A., Bhem, C. A. (1987). "Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus*." The New Phytologist **106**: 139-146.

Lindberg, S. E., Jackson, D. R., Huckabee, S. A. Janzen, J. A., Levin, M. J., Lund, J. R. (1979). "Atmospheric emission and plant uptake of mercury from agricultural soils near the Almaden mercury mine." Journal of Environmental Quality **8**: 572-578.

Lodenius, M. (1994). "Mercury in macrofungi: natural or anthropogenic." *In* Proceeding

of EnvironmentalConyman. 6th International Conference Delphi, Greece. pp. 244-246.

Loar, J. M. (2004). *State of East Fork Poplar Creek: Status of ecological recovery*. U.S. Department of Energy Mercury Workshop. Oak Ridge.

MacFarlane, E. W. (1956). "Cytological conditions in root dip meristem after gross antagonism of phenylmercuric poisoning." Experimental Cell Resources **5**: 375-385.

Magos, L., Clarkson, T. W. (1972). "Atomic absorption determination of total inorganic and organic mercury in blood." Journal of the Association of Official Analytical Chemistry **5**: 966-971.

Millhollen, A. G., Gustin, M.S. Obist, D. (2006). "Foliar mercury accumulation and exchange for three tree species." Environmental Science & Technology **40**: 6001-6006.

Mishra, A., Choudhuri, M. A. (1998). "Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth indices in tow rice cultivars." Water, Air, and Soil Pollution **114**: 339-346.

Mishra, S., Dubey, R. S. (2006). "Heavy Metal Uptake and Detoxificiation Mechanism in Plants." International Journal of Agricultural Research **1**(2): 122-141.

Munthe, H., Iverfeldt, A. (1995). "Mechanisms of deposition and mercury and methylmercury to coniferous forests." Water, Air, and Soil Pollution **80**: 363-371.

National Atmospheric Deposition Program (2009). NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr. Champaign, IL 61820, National Atmospheric Deposition Program (NRSP-3).

Nriagu, J. O., Pacyna, J. M. (1988). "Quantitative assessment of worldwide contamination of air, water and soils by trace metals." Nature **333**: 134-139.

Oda, C. E., Ingle, J. D. (1981). "Continuous flow cold vapor atomic absorption determination of mercury." Analytical Chemistry **53**: 2030-2033.

Pacovsky, R. S. (1986). "Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans." Plant and Soil **95**: 379-388.

Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., Garvin, D. F., Eide, D. Kochian, L. V. (2000). "The molecular physiology of heavy metal transport in the Zn / Cd hyperaccumlator *Thlaspi caerulescens*." Proceedings of the National Academy of Sciences of the United States of America **97**(9): 4956-4960.

Raven, P. H., Evert, Ray F., Eichhorn, Susan E. (2005). Biology of Plants. New York,

NY, W. H. Freeman and Company.

Revis, N. W., Osborne, T. R., Holdsworth, G., Hadden C. (1989). "Distribution of mercury species in soil from a mercury-contaminated site." Water, Air, and Soil Pollution **45**: 105-113.

Rogers, R. D., Williams, S. E. (1986). "Vesicular-Arbuscular Mycorrhiza: Influence on Plant Uptake of Cesium and Cobalt." Soil Biology and Biochemistry **18**: 371-376.

Sabiha-Javied, M. T., Khalid, S. (2008). "Heavy metal pollution from medical waste incineration at Islamabad and Rawalpindi, Pakistan." Microchemical Journal **90**: 77-81.

Science Application International Corporation (SAIC) (1994). East Fork Poplar Creek-Sewer Line Beltway Remedial Investigation Report: Addendum. Oak Ridge, TN, U.S. Department of Energy.

Schroeder, W. H., Munthe, J. (1998). "Atmospheric Mercury- An Overview." Atmospheric Environment **32**: 809-822.

Schutzendubel, A., Polle, A. (2002). "Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization." Journal of Experimental Botany **53**: 1351-1365.

Skoog, D. A., Holler, F. J., Nieman, T. A. (1998). Principles of Instrumental Analysis. Orlando, FL, Harcourt Brace & Company.

Theodorakis, C., Swartz,C., Rogers., W., Bickham, J., Donnelly, K., Adams, S. (2000). "Relationship between genotoxicity, mutagenicity, and fish community structure in a contaminated stream." Journal of Aquatic Ecosystem Stress and Recovery **7**: 131-143.

Trost, P. B., Bisque, R. E. (1970). Distribution of mercury in residual soils. In: Environmental Mercury Contamination, Ann Harbor Science Publishers Inc: 178-196.

Tsai, Y., Olson, B. (1990). "Effects of Hg^{2+}, CH_3-Hg^{+}, and temperature on the expression of mercury resistance genes in environmental bacteria " Applied and Environmental Microbiology **56**: 3266-3272.

Unestam, T. (1991). "Water repellency, mat formation, and leaf-stimulated growth of some ectomycorrhizal fungi." Mycorrhiza **1**: 13-20.

USEPA (1989). "National priorities listed for uncontrolled hazardous waste sites." United States Environmental Protection Agency Federal Register **54**(223): 48184-48189.

Van Bohemen, H. D., Janssen Van De Laak, W. H. (2003). "The Influence of Road

Infrastructure and Traffic on Soil, Water, and Air Quality." Environmental Management **31**: 50-68.

Williams, L. E., Pittman, J. K., Hall, J. L. (2000). "Emerging mechanisms for heavy metal transport in plants." Biochimica et Biophysica Acta (BBA)- Biomembranes **1465**(1-2): 104-126.

Wilken, R. D., Hintelmann, H. (1992). "Mercury and methlymercury in sediments and suspended particles from the river Elbe, North Germany." Water, Air and Soil **56**: 427-437.

www.ananuclear.org (2009). Oak Ridge Reservation, Alliance for Nuclear Accountability.

PART II
EFFECTS OF MERCURY ON TREE GERMINATION, DIVERSITY AND MYCORRHIAZE ALONG EAST FORK POPLAR CREEK, ANDERSON AND ROANE CO., TENNESSEE

Sharon R. Jean-Philippe, Jennifer A. Franklin*, David Buckley, Karen Hughes, and Jeremie Damay

University of Tennessee, Department of Forestry, Wildlife and Fisheries, 274 Ellington Plant Science Building, Knoxville, Tennessee 37996-4563

*Address correspondence to author. Phone: 865-9742724; email: jafranklin@utk.edu

Abstract

In the 1950s and 1960s, a large amount of mercury and other heavy metals were discharged into East Fork Poplar Creek from the Y-12 Plant Site in Oak Ridge Tennessee U.S.A. In this study, we conduct a field study along East Fork Poplar Creek to examine the long term effectS of heavy metal pollutants on tree diversity, mycorrhizal fungi presence and abundance and soil composition. Using historical soil data obtained from Science Application International Corporation (SAIC), three mercury levels, low (0-50 mg kg^{-1}), medium (50-200 mg kg^{-1}) and high (> 200 mg kg^{-1}) were used to stratify seventeen randomly selected plots. Trees > 2.54 cm in diameter within each plot were inventoried and diversity was assessed. Soils were collected and analyzed for endomycorrhizal and ectomycorrhizal presence and abundance. Total soil mercury concentration along with 23 other elements, was determined. We also assessed the inhibitory effects of methyl mercury chloride (CH_3HgCl) and mercuric nitrate $Hg(NO_3)_2$ on seed germination of four tree species: *Acer rubrum, Platanus occidentalis, Pinus taeda,* and *Pinus echinata*. Total mercury concentration ranged from 0.70 to 758.65 mg kg^{-1} for the 17 plots sampled. Over twenty tree species were identified and mycorrhizal fungi were present in all plots except one. Under *in vitro* conditions, as mercury

concentration increased above 100 mg kg^{-1}, germination of all species exposed decreased, with *P. echinata* being the least sensitive. Germination was inhibited more when seeds were exposed to methyl mercury chloride than mercuric nitrate. These results suggest that acute mercury exposure may influence forest composition more strongly than chronic mercury exposure, as a result of differential effects on germination.

Keywords: Mercury; Endomycorrhize; Ectomycorrhize; Tree diversity; Heavy metals

Introduction

Mercury (Hg) transport and distribution in the environment originate from natural (i.e. outgassing, wild fires, geothermal surfaces or volcanoes) and anthropogenic sources (i.e. fossil fuels, smelting lead, copper and zinc ores) (Boening, 2000; Schroeder and Munthe, 1998; Nriagu and Pacyna, 1988; Revis, 1989). Mercury in soil is firmly bound to organic matter or precipitated as sulphide, and found in trace concentrations in soil solution (Schuster, 1991). Schroeder and Munthe (1998) reported that roughly 75% of total soil Hg was bound to organic matter in a forest soil. In contaminated soils near a chlor-alkali plant, Hg was firmly bound to soil constituents with only a small amount of Hg found in the soil solution (Biester et al., 2002). Essential metals such as copper, iron, calcium, potassium, magnesium, sodium, chromium, and manganese are important catalysts in biochemical reactions, act as protein structure stabilizers and maintain osmotic balance (Bontidean et al., 2004), while non-essential metals, such as mercury, cadmium and lead have no biological role. The occurrence of certain metal species in soil environments depends on several factors: ionic potential, organic matter content, oxides, cation exchange capacity and pH.

Plants influence the biochemistry and distribution of metals in soil fractions. Mechanisms by which plant roots and the rhizosphere change the soil environment include change in soil pH through the release of H^+, or excretion of exudates (i.e. organic acids or phytosiderophers) which sequester metals in the rhizosphere, thereby increasing metal concentrations in the soil solution and plant uptake (Marschner, 1995). Mercury absorption by plant roots is low (Lodenius, 1994; Gilmour and Miller, 1973; Lindberg et

al., 1979), with restricted transport of Hg observed in the phloem. Since Hg is not required for plant growth, there probably is not a specialized transporter for Hg. Therefore, Hg accumulation within plant components is through root cells, via transporters for essential cations (Kim et al., 2002).

The soil microbial community plays an important role in energy flow, nutrient cycling, soil structure stability, and decomposition processes. Fungi are important to the community adding diversity, uniqueness and antiquity but also they provide symbiotic association within the terrestrial environment. Through these symbiotic associations several beneficial mutualisms evolved, endomycorrhizal and ectomycorrhizal, ericoid and orchid mycorrhizal associations. Between 80 and 92% of surveyed land plant species and families are mycorrhizal, with endomycorrhizae being the predominate mycorrhiza (Wang and Qui, 2006).

In the 1950's [particularly 1956-1958] and early 60's, over 1,080 metric tons of Hg was released into the environment through the soil, with a resulting discharge into East Fork Poplar Creek (EFPC) from the U.S. Department of Energy Y-12 site in Oak Ridge, Tennessee U.S.A (Revis et al., 1989; Han et al., 2006). Mercury was used to separate lithium isotopes during the production of nuclear weapons (Han et al., 2006). This 15-mile long creek flows through the city of Oak Ridge in Anderson County and empties into the Clinch River in Roane County, TN. Levels of total Hg in the floodplain soils along the creek ranged, in 1984, from 0.5 to 3000 mg kg^{-1} (Revis et al., 1989). In addition to Hg discharged into unlined landfills, ponds and streams, other significant contaminates such as arsenic, chromium, lead, nickel, silver, zirconium, uranium,

technetium and plutonium have been released from facilities associated with The Department of Energy (Kelsh and Parsons, 1997). Following remediation (i.e. pump-and-treat, in-situ anaerobic bioremediation and hydrofracture) of EFPC and other tributaries by the U.S. Department of Energy and Science Application International Corporation (USEPA, 1989; SAIC, 1994), total soil mercury ranged between 0 to 200 mg kg^{-1} along transects with some areas > 200 mg kg^{-1}. Forests of the EFPC floodplain have therefore received an acute dose of mercuric nitrate and elemental mercury during the 1950s, followed by the chronic presence of Hg exposure over the past 50 years. Although several studies have investigated effects of Hg and other toxic metals on aquatic populations (Burger et al., 2005), soil bacteria diversity (Tsai and Olson, 1990), mercury bioavailability in soils (Han et al., 2006) and aquatic plants (Stewart et al., 1992), little is known regarding the long term effects of mercury contamination on forest composition through initial influences on germination, and ongoing influences on trees and endomycorrhizal and ectomycorrhizal associations. We first conducted a field study to investigate tree diversity, documented mycorrhizal presence and abundance, and determined physiochemical composition of the soils along EFPC mercury concentration gradients along, post-heavy metal exposure. We tested whether tree diversity and endomycorrhizal and ectomycorrhizal presence and abundance decreases as total soil mercury concentration increases. W also evaluated the ability of several tree species (*Platanus occidentalis, Acer rubrum, Pinus echinata* and *Pinus taeda*) to germinate in various mercury solutions, to determine lethal and non-lethal concentrations for each

species. We finally tested whether germination rate for each species decreases as mercuric concentration increases.

Methods

During June 2007, three blocks B1 (N 36° 00.52′, W 084° 14′), B2 (N 36° 00.33′, W 084° 16.82′) and B3 (N 35° 95.243′ W 084° 38.19′) were established within the floodplain of East Fork Poplar Creek (EFPC) perpendicular to the creek bank (Figure 2.1). The Natural Resources Conservation Service classified soils along EFPC as Inceptisols. These Newark silt loams are somewhat poorly drained and are moderately acid to moderately alkaline. Historical data for soil total Hg concentration along EFPC obtained from Science Application International Corporation (SAIC, 1994), was used to establish plots along the creek. Three Hg contamination levels, low (0-50 mg kg^{-1}), medium (50-200 mg kg^{-1}) and high (>200 mg kg^{-1}) were established within each of the blocks, and circular plots were randomly placed within each of these areas. Plot size was 168.1 m^2 (0.017 hectare) with 3 plots each for low and medium levels and one high level within Block 1 and Block 3. Block 2 was smaller in area and had one of each contamination level represented. The high/medium/low classification of some plots was later changed based on the results of our analysis. Due to remediation efforts along EFPC during the late 1980's, there were few areas to establish high plots. Within each plot, species and diameter were inventoried for woody plants measuring > 2.54 cm diameter at breast height. Soils were sampled during June and October 2007 and June and October 2008. Three, 30.48 cm soil cores were randomly collected from each plot. The three soil samples collected from each plot were mixed together in the field, placed in storage bags

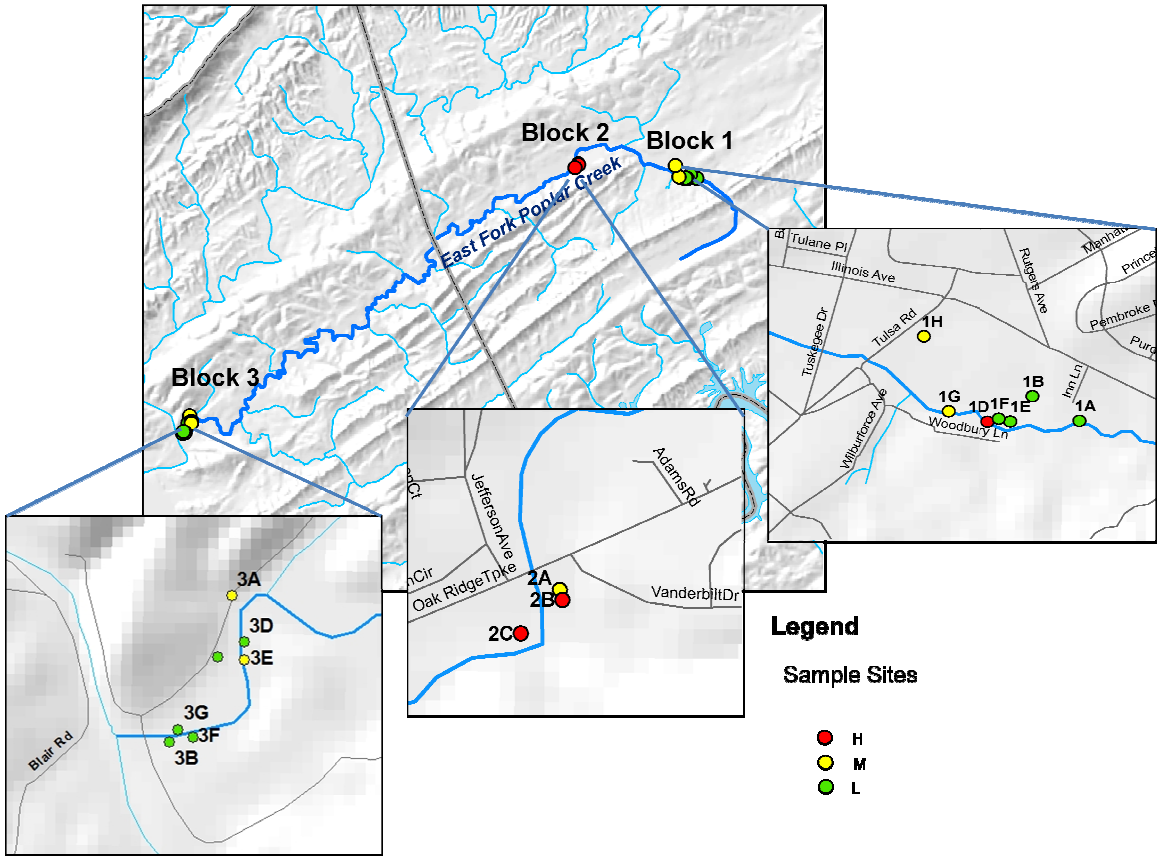


Figure 2.1. Study areas located along East Fork Poplar Creek in Anderson and Roane Counties Tennessee, ArcMap 8.0. Mercury level shown is based on actual total soil Hg (see Table 2.1).

and taken back to the lab where soil samples were placed in the freezer until analysis. Sub-samples from this heterogeneous mixture were used to identify total mercury analysis and physiochemical composition of EFPC soils.

Mycorrhizal counts were done for June and October 2007 and June and October 2008. Following the trypan staining methods of Augé and Moore (2005), 136 g of soil sample from each of the 17 plots were homogenized in 3% Calgon (sodium pyrophosphate decahydrate) overnight. Roots were washed in deionized water and placed

in cassettes. Root samples were immersed in 10% potassium hydroxide, boiled 3 times for 20 minutes each to remove pigments from roots and rinsed 3 times in tap water and 1 time in deionized water. Roots were then immersed in 3% hydrogen peroxide for 1 hour, rinsed 3-5 times in tap water and 1 time in deionized water. Roots were acidified in 2% HCl for 1.5 hours and then immersed in 0.05% trypan blue stain for 1 hour. Roots were rinsed 2-3 times with distilled water and immersed in lactoglycerol solution (equal volume of 85% lactic acid, glycerol and distilled water) until analyzed. Additional sub-samples of roots were fixed in FAA (formalin-acetic acid-alcohol) to quantify ectomycorrhizal presence (Augé and Moore, 2005).

Roots were plated on slides and counted using a compound light microscope, starting at the bottom right end of each slide moving right to left; roots that came into view were assessed for presence or absence of vesicles or arbuscules (which indicate the presence of endomycorrhizae) until a hundred counts were obtained. Root length colonization percentage was obtained by dividing the number of colonized roots by total number of roots. To assess ectomycorrhizae, FAA fixed roots were placed in a glass petri dish and counted using the grind line intersection method (Agerer, 1991).

All soil samples were air dried for 2 days and finely ground and sieved through a 250 µm (60 –mesh) sieve. Soils were air dried to prevent volatization of certain mercury species that occurs above 30 ˚C. Total elemental analysis was determined by following Nadkarni (1984), using microwave oven digestion with modifications. Total elemental analysis of digested soil samples was analyzed by ICP. Exchangeable Ca, K, Mg, Pb, Cd, Mn, Fe, Zn, Ni and Cu were determined by extraction with 1 N ammonium acetate (pH

7.0) by addition of concentrated ammonium hydroxide, followed by analysis by inductively coupled atomic emission spectrometry (ICP). Cation exchange capacity (CEC) was determined by extraction with 2 N ammonium followed by distillation. Total nitrogen (N) and carbon (C) was determined on a Thermo Eager 3000 analyzer. Soil pH was measured by mixing 1:1 volume by weight soil and deionized water into a 50 ml falcon tube. Samples were brought up to final volume (40 ml) with additional deionized water, shaken for on one minute and allowed to settle for one hour before pH was read. Soil samples were analyzed for total mercury concentration by cold vapor atomic absorption spectroscopy (CVAAS) by Western Kentucky University, Institute of Combustion Science and Environmental Technology. Only soil samples collected in October of 2008 were analyzed for total mercury.

Differences between tree species diversity in plots within blocks were assessed by the Shannon diversity index, $H = -\sum^{s}_{i=1} p_i (\ln p_i)$, where s = number of species, p_i fraction of the entire population made up of species i. The Spearman rho rank-based non-parametric test was used to correlate endomycorrhizae, ectomycorrhizae count, tree species diversity, tree diameter, basal area, cation exchange capacity (CEC), pH, average Ca, C, N, Hg, Al, Ba, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Nd, Ni, P, Sr, Ti, V, Zn, Pb, Rb, and S.

American sycamore (*Platanus occidentalis*), red maple (*Acer rubrum*) and pine all occur within the floodplain areas of EFPC. From the seventeen vegetation plots inventoried, sycamore is the only species that occurs within all plots. Also red maple and one unknown pine species only occurred in vegetation plots where total soil mercury

concentration was < 50 mg kg^{-1}, so the effects of lethal and non-lethal mercuric solution on germination of four different tree species were investigated.

Seeds for red maple, sycamore, loblolly pine (*P. taeda*) and shortleaf pine (*P. echinata*) were obtained from Sheffield Seed Company, New York. Seeds were sterilized for three minutes in a 10% bleach solution, and soaked in deionized water for 1 hour. Twenty five seeds of red maple and the two pines species were placed on larger Petri-plates lined with filter paper, while 50 seeds of sycamore were placed on smaller Petri-plates lined with filter paper.

Stock solutions (500 mg kg^{-1}) of mercuric nitrate ($Hg(NO_3)_2$) and methyl mercury chloride (CH_3HgCl) were prepared by mixing 0.27 grams of each compound in 540 mL of deionized water. The stock solution was further diluted to obtain final concentrations of 5, 10, 50, 100, and 500 mg kg^{-1} solutions. Depending on plate size, either 3 or 10 mL of mercuric solution was applied.

Each species (sycamore, red maple, loblolly and shortleaf) were germinated in each of the two mercuric solutions (treatment), $Hg(NO_3)_2$ and CH_3HgCl. Each treatment was replicated four times. Seeds were germinated *in vitro* with two different incubation environments based on optimum conditions (Bonner, 2008; Krugman and Jenkinson, 2008; Zasada and Strong, 2008); sycamore and pine species were placed in an incubator with 8 hours of light at 30°C and 16 hours of dark at 20°C; for red maple 8 hours of light at 15°C and 16 hours of dark at 5°C. Germination percentage was recorded on day 7, 9, 12, 22, 37, and 44 for sycamore seeds and day 7, 13, 15, 22, 38, and 44 for pine species.

Viable seedlings from *Pinus sp.* and *P. occidentalis* were transplanted into sterilized 8" pots containing 400 g of sterile 2:1 vermiculite/sand media.

Analysis of variance (ANOVA) was used to test for mean differences between block (experiment) by treatment interaction for each tree species for the germination study. ANOVA repeated measures combining the 4 replications using a Randomized Complete Block Design was used to test for differences in mean germination rate by tree species, mercury species, and mercury concentration using day as a repeated measure. All data analysis was performed by SAS 9.1.3.

Results

Total mercury concentration ranged from 0.70 to 758.65 mg kg^{-1} in the seventeen plots sampled (Table 2.1). Vegetation plots were initially established based on soil mercury sampling data from SAIC. Several plots were either lower (1A, 1E, 2A, 3C, 3E, and 3G), or higher (1H, 2B, and 2C) then what was initially assigned. ICP analysis of EFPC soils identified twenty one major and trace elements (Table 2.2). Based on Spearman's rho correlation coefficients, carbon (.85, $p< .000$), copper (.956, $p< .000$) iron (.919, $p < .000$) and rubidium (.907, $p< .000$), titanium (.623, $p< .008$) and vanadium (.716, $p<.001$) were correlated with soil total mercury concentration. Tree species composition within the floodplain area of EFPC is diverse, but the tree species diversity was not correlated to soil total mercury concentration (-.120 $p> 0.64$). Twenty different species were identified in established vegetation plots along EFPC. The most abundant tree species present within established plots along the creek were *Oxydendrum arboreum* (sourwood), *Liriodendron tulipifera* (yellow poplar), *Acer rubrum* (sugar maple) and

Table 2.1. Total soil mercury concentration of EFPC soils.

Block/Plot	Initial Hg-level	Measured Hg-level	Avg. mg Hg kg^{-1}
1A	medium	low	38.7
1B	**low**	**low**	0.945
1D	**high**	**high**	216.2
1E	medium	low	37.95
1F	**low**	**low**	6.93
1G	**medium**	**medium**	113.4
1H	low	medium	52.05
2A	high	medium	82.95
2B	medium	high	212.55
2C	low	high	758.65
3A	**medium**	**medium**	74.45
3B	**low**	**low**	4.505
3C	medium	low	0.705
3D	**low**	**low**	43.8
3E	high	medium	76.65
3F	**low**	**low**	6.33
3G	medium	low	25.05

Initial Hg levels low (>50 mg kg^{-1}), medium (> 200 mg kg^{-1}) and high (>200 mg kg^{-1}) were assigned based on data obtained from SAIC.

Total soil Hg was obtained from October 2008 soil samples.

Acer negundo (box elder). These species do not drastically differ in preferred growing areas but they do have varying shade tolerances (Canham, 1989). Two of the least diverse plots, where only one species was present, had low (> 50 mg kg^{-1}) to medium (> 200 mg kg^{-1}) soil mercury concentrations. Only one species, American sycamore occurred within all plots established along the creek.

The abundance of endomycorrhizal (-.257, p>0.30) and ectomycorrhizal (-0.5, p>0.02) fungi were not correlated with total soil mercury concentration, or elemental analysis, but ectomycorrhizal abundance was positively correlated with species diversity

Table 2.2. Summary of analytes from EFPC soils.

	Minimum	Maximum	Mean
Al_{ppm}	57.05	127.15	94.84
Ba_{ppm}	61.15	114	83.62
$C_{total\ mg\ g\text{-}1}$	0.63	2.34	1.51
Ca_{ppm}	59.3	355.2	169.92
Cd_{ppm}	0.02	0.05	0.027
Cr_{ppm}	0.04	0.19	0.097
Cu_{ppm}	0	0.25	0.096
Fe_{ppm}	13.88	58.65	40.76
Hg_{ppm}	0.7	758.65	103.04
K_{ppm}	14.43	31.19	19.43
Mg_{ppm}	7.74	25.45	16.19
Mn_{ppm}	0.28	3.87	1.51
$N_{total\ mg\ g\text{-}1}$	0.04	0.13	0.092
Na_{ppm}	4360	9300	5315.4
Nd_{ppm}	0.02	0.05	0.034
Ni_{ppm}	0	0.19	0.058
P_{ppm}	0.48	2.05	1.21
Pb_{ppm}	0.05	0.14	0.11
Rb_{ppm}	0.04	0.16	0.113
S_{ppm}	0.16	0.59	0.39
Sr_{ppm}	0.04	0.08	0.048
Ti_{ppm}	2.47	4.23	3.31
V_{ppm}	0.04	0.12	0.082
Zn_{ppm}	0.02	0.4	0.177
CEC	0.13	12.09	7.77
pH	5.66	8.04	7.12

*CEC (meq/ 100g soil) and pH were analyzed for 16 soil samples (except 2A).

Table 2.3. Number of tree species and mycorrhizal fungal presence within the plots (n=17) sampled along EFPC.

Block	Tree Diversity	Mycorrhizal Presence Endo	Ecto
1A	1.65	Yes	Yes
1B	1.73	Yes	Yes
1D	1.53	Yes	Yes
1E	1.08	Yes	Yes
1F	1.7	Yes	Yes
1G	1.95	Yes	Yes
1H	1.2	No	Yes
2A	0.95	Yes	Yes
2B	0.69	No	No
2C	0.48	No	Yes
3A	1.49	Yes	Yes
3B	0.73	Yes	Yes
3C	0.45	Yes	Yes
3D	1.06	Yes	Yes
3E	0	Yes	Yes
3F	1.45	Yes	Yes
3G	1.32	Yes	Yes

(.722, $p< 0.001$) as determined by Shannon diversity index and negatively correlated with diameter (-.717, $p < 0.001$). The average tree diameter ranged from 8.0 to 20.4 cm in Block 1, 19.5 to 34.2 cm in Block 2, and 11.1 to 31.7 cm in Block 3. Both endomycorrhizal and ectomycorrhizal fungi were present in all plots sampled except plots 1H, 2B and 2C (Table 2.3). Species present in these plots: *Aesculus flava* (yellow buckeye), *Ulmus rubra* (slippery elm), *Liquidambar styraciflua* (sweet gum), *Juniperus virginiana* (Eastern red cedar), *Lindera benzoin* (spicebush), all found in plot 1H, yellow-poplar (2B), box elder (1H, 2B and 2C), sourwood (1H and 2C), and *Prunus serotina* (black cherry) (2C) all reportedly are endomycorrhizal fungi associated tree species, but endomycorrhizal-fungi were not observed in the soil samples taken from these plots, but

possible are present in these plot. The two plots with the highest soil mercury concentration 1D (216.2 mg kg^{-1}) and 2C (758.65 mg kg^{-1}) both had ectomycorrhizae present in the roots collected from these plots.

Red maple did not germinate at all in any mercuric or control solution. Both pine species germinated in $Hg(NO_3)_2$ and CH_3HgCl solutions $\leq$ 500 ppm, and $\leq$ 100 ppm, respectively (Figure 2.2 and 2.3). American sycamore germinated in $Hg(NO_3)_2$ and CH_3HgCl solutions $\leq$ 10 ppm (Figure 2.4). Tukey's test for mean differences resulted in a significant interaction for loblolly pine ($P < 0.0002$) but not for sycamore and shortleaf pine for block (experiment) by treatment. The 4-way interaction was significant ($P < 0.001$) so each tree species was evaluated separately for a 3-way interaction (mercury species*concentration*day). The data meet normality assumptions but due to unequal variances between treatment levels a rank transformation was conducted (Table 2.4). The mean separation and corresponding letter results are presented in Tables 2.5-2.7.

When comparing the effects of mercuric compound on mean germination by experimental days for each species, sycamore did not differ significantly. Shortleaf pine differed significantly at concentrations 0, 10 - 500 mg kg^{-1} for day 7 and 0, 5, 50 and 500 for day 12 and day 22 at 50-500 mg kg^{-1} for CH_3HgCl, but for $Hg(NO_3)_2$ days 7 - 44 did not differ significantly at any concentration. Loblolly pine differed significantly at concentration 0, 50 and 100 ppm for day 7, and differed at all concentrations on day 12 for CH_3HgCl. But for $Hg(NO_3)_2$, day 7 differed from days 12 - 44. When comparing the effects of mercuric compound on germination percentage at different concentrations for each tree species, sycamore differed at 5 mg kg^{-1} during the exposure days of 22 - 44 and

Table 2.4. Effect of mercury*concentration*day on germination rate for each species

Specie	Source	Num DF	Den DF	F-value	Pr > F
Plantanus occidentalis	Mer*Con*Day	20	144	8.41	< .0001
Pinus echinata	Mer*Con*Day	20	144	6.87	< .0001
Pinus taeda	Mer*Con*Day	20	144	2.19	< .0042

Table 2.5. *Platanus occidentalis* mean separation for mercury species by concentration by day

CH_3HgCl Day	7	12	22	37	44
Concentration (mg kg^{-1})					
0	18.2 ± 3.1^{A}	19.2 ± 2.2^{A}	0.5 ± 1.0^{FG}	0.2 ± 0.5^{FG}	0.2 ± 0.5^{FG}
5	17.5 ± 3.7^{A}	19.0 ± 1.4^{A}	6.7 ± 4.6^{C}	4.5 ± 5.2^{CD}	4.2 ± 5.0^{CDE}
10	10.5 ± 2.0^{B}	11.0 ± 3.4^{B}	1.0 ± 1.4^{FG}	0.5 ± 1.0^{FG}	0.5 ± 1.0^{FG}
50	1.7 ± 0.5^{DEFG}	3.0 ± 1.1^{DEF}	0	0	0
100	1.7 ± 2.0^{DEFG}	2.2 ± 2.6^{DEFG}	0	0	0
500	1.25 ± 0.5^{EFG}	1.0 ± 0.8^{FG}	0	0	0
$Hg(NO_3)_2$ Day	7	12	22	37	44
Concentration (mg kg^{-1})					
0	14.5 ± 3.7^{AB}	13.5 ± 3.1^{B}	1.7 ± 1.7^{DE}	1.7 ± 1.7^{DE}	1.7 ± 1.7^{DE}
5	17.0 ± 3.9^{A}	16.0 ± 4.2^{AB}	2.7 ± 0.5^{DE}	3.0 ± 0.8^{DE}	3.0 ± 0.8^{DE}
10	13.0 ± 2.7^{B}	14.5 ± 5.5^{AB}	4.2 ± 2.7^{CD}	4.0 ± 2.5^{CD}	4.0 ± 2.5^{CD}
50	6.5 ± 5.0^{C}	1.2 ± 1.5^{DE}	0	0	0
100	3.5 ± 1.7^{CD}	3.5 ± 1.7^{CD}	0	0	0
500	2.7 ± 1.7^{DF}	2.5 ± 1.9^{DE}	0	0	0

Means with the same letter labels are not significantly different.

Table 2.6. *Pinus echinata* mean separation for mercury species by concentration by day

CH_3HgCl	Day	7	12	22	37	44
Con						
0		8.2 ± 1.2^{D}	20.2 ± 2.8^{AB}	15.5 ± 5.0^{C}	14.0 ± 4.5^{C}	14.0 ± 4.5^{C}
5		7.7 ± 1.2^{D}	21.25 ± 2.7^{A}	16.2 ± 5.7^{C}	15.7 ± 6.1^{C}	15.0 ± 5.7^{C}
10		6.0 ± 1.8^{DEF}	20.5 ± 0.5^{AB}	14.7 ± 0.9^{C}	14.2 ± 2.0^{C}	14.2 ± 1.7^{C}
50		3.5 ± 2.0^{EFG}	17.25 ± 2.2^{BC}	7.5 ± 1.7^{D}	4.7 ± 1.5^{DEF}	1.7 ± 0.9^{G}
100		7.0 ± 1.6^{DE}	21.5 ± 1.2^{A}	2.7 ± 0.9^{FG}	0.7 ± 1.5^{G}	0.2 ± 0.5^{G}
500		0	2.7 ± 0.9^{FG}	0	0	0
$Hg(NO_3)_2$	Day	7	12	22	37	44
Con						
0		7.5 ± 2.3^{EFGH}	21.75 ± 2.3^{A}	11.75 ± 4.0^{BCD}	10.0 ± 4.5^{BCDE}	10.0 ± 4.5^{BCDE}
5		7.2 ± 3.3^{EFGH}	19.7 ± 1.7^{A}	8.7 ± 5.3^{DEFG}	$8.0 \pm 5.6D^{EFGH}$	$7.7 \pm 5.5D^{EFGH}$
10		5.5 ± 1.7^{FGH}	19.7 ± 1.7^{A}	13.2 ± 1.7^{B}	13.2 ± 1.7^{B}	13.0 ± 1.6^{BC}
50		5.2 ± 0.9^{GH}	20.5 ± 1.9^{A}	13.0 ± 2.9^{BC}	12.2 ± 2.9^{BC}	13.0 ± 2.9^{BC}
100		4.5 ± 1.2^{H}	20.5 ± 2.0^{A}	10.7 ± 1.7^{BCDE}	9.2 ± 1.2^{CDEF}	8.7 ± 1.7^{DEFG}
500		4.5 ± 1.0^{H}	19.2 ± 3.8^{A}	11.75 ± 2.6^{BCD}	11.0 ± 2.4^{BCDE}	10.7 ± 2.2^{BCDE}

Means with the same letter labels are not significantly different.

at concentration 500 mg kg^{-1} on day 7 for CH_3HgCl but for $Hg(NO_3)_2$ differed at 5, 50 and 500 mg kg^{-1} on day 7. Shortleaf pine differed at 50 mg kg^{-1} during exposure days 7 - 22 for CH_3HgCl but for $Hg(NO_3)_2$ at all concentration day 12 differed from all other exposure days. Loblolly pine differed at 10 mg kg^{-1} during exposure days 12 & 22, at 50 mg kg^{-1} for days 7 - 37 and 100 mg kg^{-1} for day 12 for CH_3HgCl but for $Hg(NO_3)_2$ concentration 0 – 50 mg kg^{-1} differ from 100- 500 mg kg^{-1} for days 7 & 12. Figures 2.2-2.4 (a, b), show the mean cumulative germination percentage by day and concentration. Under *in vitro* conditions, as mercury concentration increased, germination decreased for all species examined. Germination was inhibited more when seeds were exposed to methyl mercury chloride than mercuric nitrate.

Table 2.7. *Pinus taeda* mean separation for mercury species by concentration by day

CH_3HgCl	Day	7	12	22	37	44
Con						
0		0.7 ± 0.5^{FGHI}	15.0 ± 2.4^{ABC}	16.0 ± 1.4^{AB}	16.0 ± 2.1^{AB}	15.7 ± 1.7^{AB}
5		1.7 ± 1.5^{FGHI}	15.5 ± 2.8^{AB}	15.2 ± 3.4^{ABC}	15.5 ± 3.1^{AB}	15.7 ± 3.1^{AB}
10		2.2 ± 0.9^{FGHI}	12.7 ± 1.7^{BD}	15.2 ± 0.9^{AC}	13.7 ± 2.0^{ABC}	14.0 ± 1.4^{ABC}
50		1.2 ± 0.5^{HI}	11.7 ± 4.4^{CD}	4.2 ± 4.2^{EF}	3.7 ± 2.3^{FG}	2.7 ± 1.7^{FGHI}
100		0.5 ± 0.5^{GHI}	7.5 ± 1.2^{E}	1.2 ± 1.8^{FGHI}	0.2 ± 0.5^{GHI}	0.2 ± 0.5^{GHI}
500		0.7 ± 0.9^{FGHI}	2.5 ± 2.5^{FGH}	0	0	0
$Hg(NO_3)_2$	Day	7	12	22	37	44
Con						
0		2.7 ± 0.9^{FG}	17.0 ± 4.4^{A}	15.5 ± 3.7^{AB}	15.0 ± 4.0^{ABC}	14.7 ± 3.8^{ABC}
5		1.5 ± 0.5^{FG}	13.0 ± 5.3^{BCD}	10.7 ± 6.8^{DE}	10.2 ± 6.9^{E}	10.2 ± 6.9^{E}
10		1.7 ± 0.9^{FG}	15.7 ± 3.3^{AB}	15.5 ± 3.7^{AB}	14.0 ± 3.3^{ABCD}	14.7 ± 3.5^{ABC}
50		1.2 ± 1.2^{FG}	13.25 ± 1.7^{BCDE}	13.7 ± 1.8^{ABCDE}	13.7 ± 1.8^{ABCDE}	14.0 ± 2.1^{ABCD}
100		0.5 ± 0.5^{G}	10.5 ± 3.3^{DE}	11.5 ± 2.0^{CDE}	11.5 ± 2.0^{CDE}	11.5 ± 2.0^{CDE}
500		0.5 ± 0.5^{G}	4.2 ± 1.2^{F}	4.2 ± 2.8^{F}	4.5 ± 2.6^{F}	4.2 ± 2.8^{F}

Means with the same letter labels are not significantly different.

Discussion and Conclusion

During the 1950's and 1960's a large quantity of mercury and other heavy metals were released into East Fork Poplar Creek in Oak Ridge, Tennessee. The release of these contaminates were at levels that were probably lethal to established seed banks, vegetation, and soil microbial populations. We first tested whether tree diversity and endomycorrhizal and ectomycorrhizal presence and abundance were negatively affected as total soil mercury concentration increased within sampled plots. No significant correlated were found between tree species richness and soil mercury concentration. The ability for heavy metals to concentrate within the soil and subsequently affect the presence of tree species depends on several factors, which include concentration and chemical form of the heavy metals.

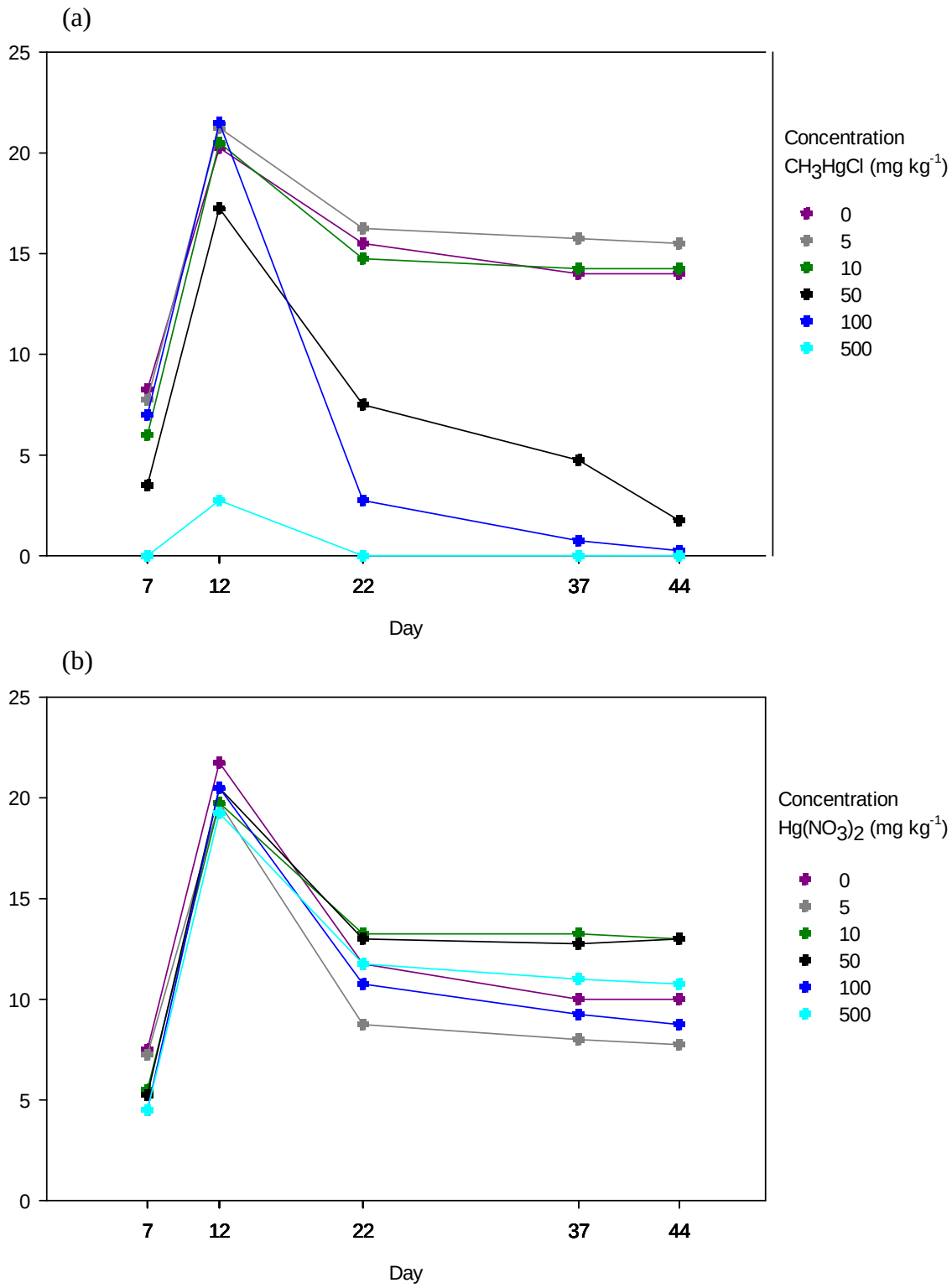


Figure 2.2. *Pinus echinata* mean germination rate by day and concentration for (a) CH_3HgCl and (b) $Hg(NO_3)_2$.

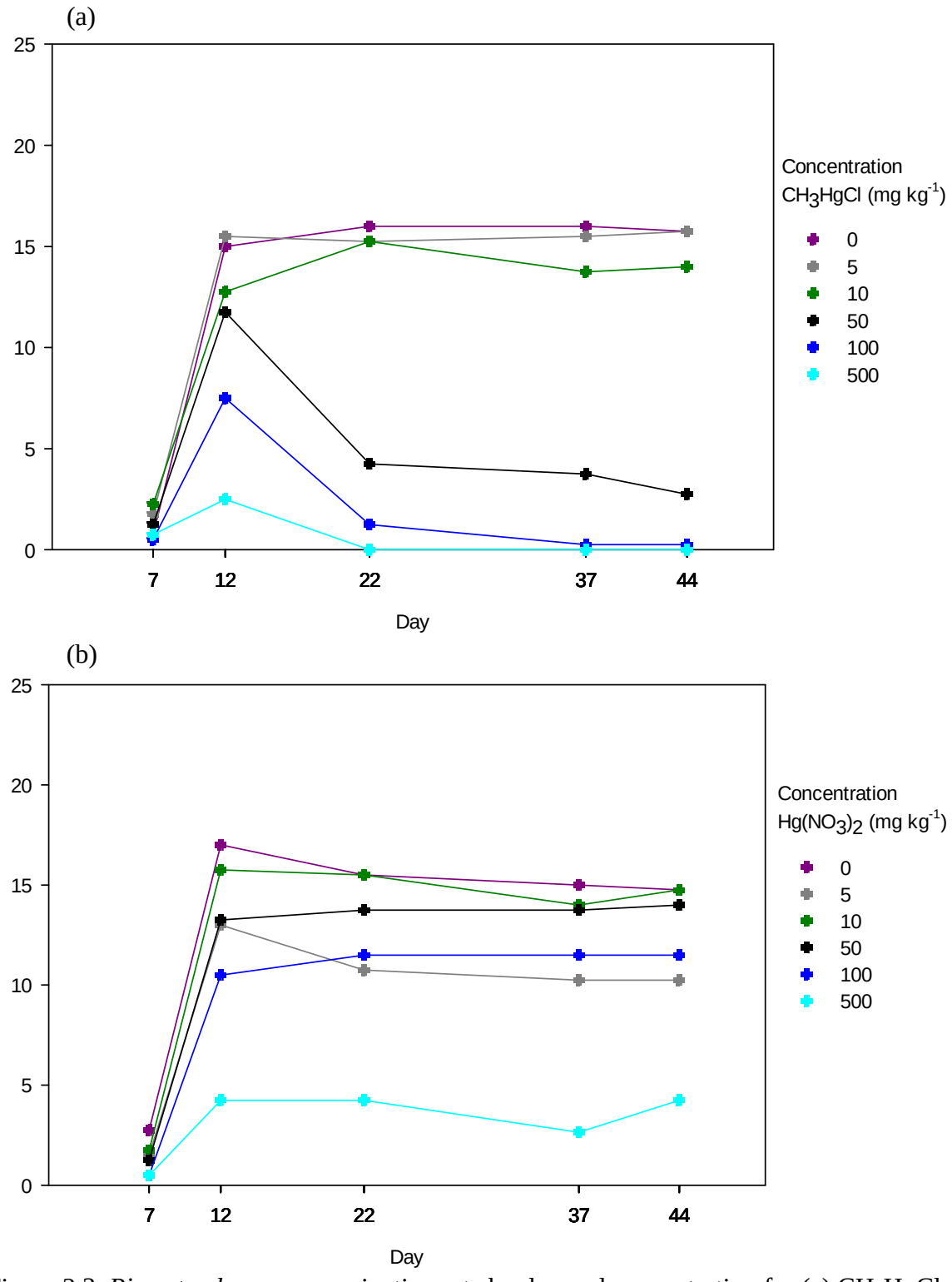


Figure 2.3. *Pinus taeda* mean germination rate by day and concentration for (a) CH_3HgCl and (b) $Hg(NO_3)_2$.

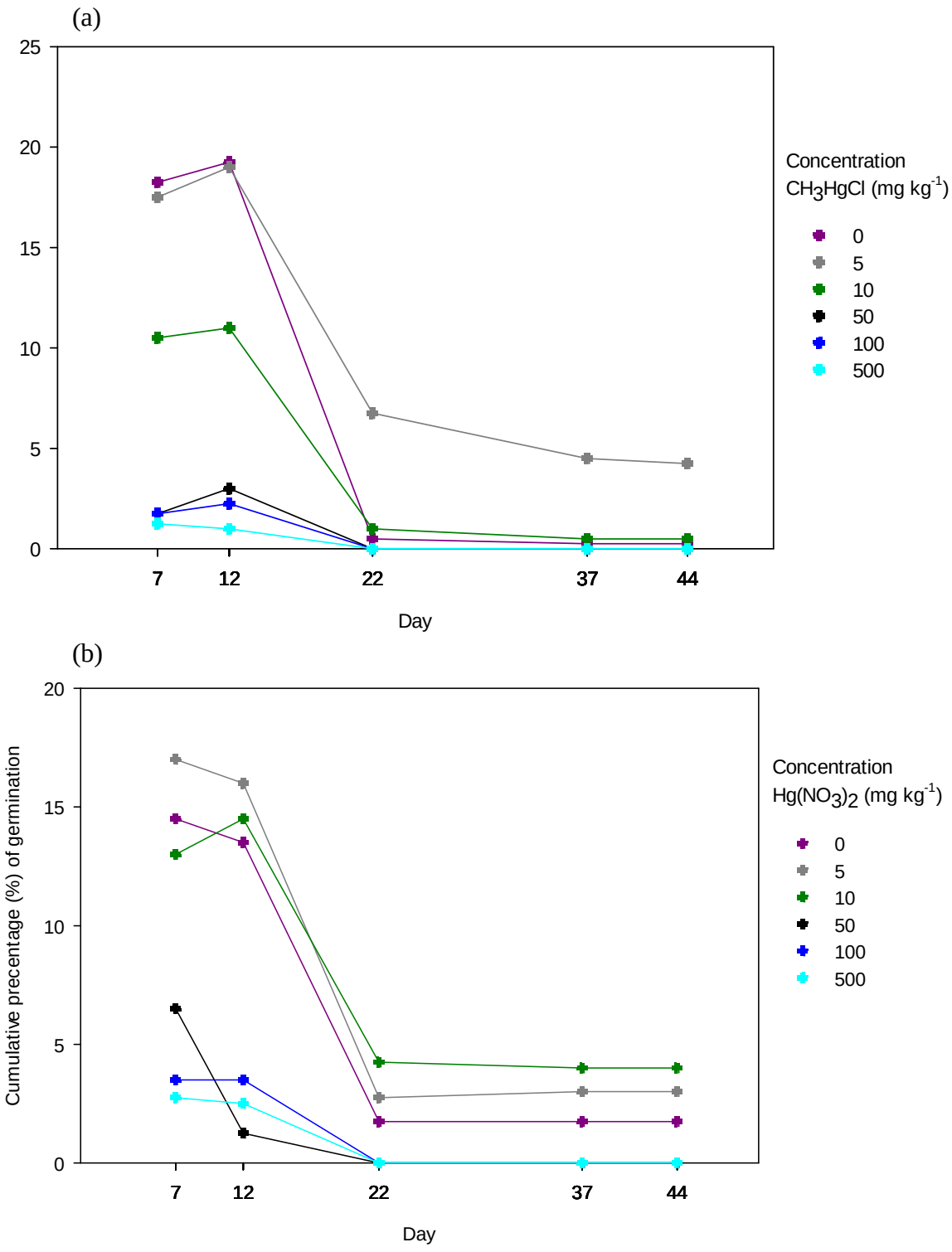


Figure 2.4. *Platanus occidentalis* mean germination rate by day and concentration for (a) CH_3HgCl and (b) $Hg(NO_3)_2$.

The primary forms of mercury released into EFPC during the 50's and 60's were mercuric nitrate and elemental mercury. It is plausible to think that from the initial release of heavy metals, woody plant responded by adapting avoidance (roots) or tolerance (sequestration or chelation) mechanisms, or their tolerance to heavy metals was enhanced through mutualistic associations shared between plant and fungi.

Soil analysis for mycorrhizal presence identified both endomycorrhizal and ectmycorrhizal fungi. The majority of tree species inventoried in our established vegetation plots are reportedly endomycorrhizal associated species. There was no significant correlation found between mycorrhizal presence and soil total mercury concentration or soil elemental content. Del Val et al., (1999) did find a negative correlation ($P < 0.001$) between arbuscular mycorrhizal fungi (AMF) spores and soil metal content. However several authors found no correlation between the metal concentration and AMF populations (Griffioen, et al., 1994; Weissenhorn and Leyval, 1994). Mycorrhizal populations have been shown to fluctuate during seasonal cycles. Also, during the two years of sampling, the area experienced a severe drought which could have influenced the presence and abundance of mycorrhiza in our analysis (Shi et al., 2002).

We did observe a positive correlation between ectomycorrhizal abundance and tree diversity but a negative correlation to tree diameter. Liang et al., (2007) found a positive correlation between tree diversity and ectomycorrhizal abundance, specifically, where non-ectomycorrhizal trees were positively correlated to ectomycorrhizal sporocarp abundance. They postulated that non-ectomycorrhizal trees created suitable habitats for

ectomycorrhizal aggregation. From our results the negative correlation between ectomycorrhizal abundance and tree diameter seem to imply that tree diameter affects mycorrhizal presence. Urcelay and Robledo (2009) found that the abundance of basidiocarps in the Andean Alder forest in Northwest Argentina was positively correlated to log diameter. So the size of the logs strongly affected the presence of basidiocarps. Tree/mycorrhizae interactions are complex in the natural forest, and this complexity is only heightened when the forest has been affected by contamination. Even though the majority of trees identified from our field study were found to have endomycorrhizal association, plot 2C had a total soil mercury concentration > 700 mg kg^{-1} and only ectomycorrhizae were present. We could hypothesize that at higher contamination levels, the abundance of fungal populations were greatly reduced due to toxicity, but that tolerant propagules never disappeared completely from the soil. Interestingly, plot 2B had a total soil mercury concentration > 200 mg kg^{-1} and neither endo- nor ectomycorrhizae were present. Besides mercury, several other heavy metals along with other physiochemical components (pH, soil type, CEC, element abundance) of the soil environment also may affect the presence of species in the environment.

The effect of heavy metals on plant/fungal populations in natural habitats varies with environmental factors (pH, soil type, metal availability), and the presence of other biological organisms (Gadd and Griffiths, 1978). The ability to adapt to polluted soils is probably not because of adaptive changes but due to intrinsic properties of species present, chemical and physical properties of the environment (Gadd, 1993). Studies have shown that the distribution pattern of plant and soil animal species near pollution sources

are severely limited due to high contamination level, but at greater distances from pollution source, species distribution increases (Helmisaair et al., 1995; Salemaa et al., 2001). Mycorrhizal fungal populations are critical during and after the release of soil contaminates because of their important role in establishment and survival of plants (Haselwandter and Dowen 1996; Miller and Jastrow 1992).

To better understand the effects on heavy metal contamination on tree species we conducted a germination study to test the lethal and non lethal limits for each species. From our germination study we can see that the ability for certain tree species to germinate in *in vitro* conditions depends on type of mercury compound, mercury concentration applied, and tree species. Our results show that at the highest concentration of mercuric nitrate solution, the two pines species germinated, and survived until the completion of the experiment (Figure 2.2 (b) and 2.3 (b)), whereas, sycamore germinated in the 50-500 mg kg^{-1} mercuric nitrate solution, but quickly declined and died by day 22 (Figure 2.4(b)). Prior to germination, sycamore seeds were soaked in water overnight. This pretreatment before mercury application may have delayed the effects of the mercuric solutions on the seeds, which resulted in their initial germination. Also, the control treatments fared poorly after day 12 and were all dead by day 22 (Figure 2.4 (b)). The effects of methyl mercury chloride on seed germination proved to be more harmful. Germinating seeds of the two pines species followed the same trend, where after day 12 at 500 mg kg^{-1} germination halted and germinates died (Figure 2.2 (a) and 2.3 (a)). The same effects were observed with sycamore, but the control treatments also declined and were all dead by day 22 (Figure 2.4 (a)). Several authors have investigated the seed

developmental stage of the plant life cycle and how essential and non-essential metal ions can affect seed developmental processes (Fargasova, 1994; Li et al., 2005; Munzuroglu and Geckil, 2002). Our results suggest that possibly the seed coat of the two pine species played an important role in protecting the embryo from heavy metal toxicity. Li et al., (2005), showed that by removing the seed coat of *Arabidopsis thaliana* seeds, isolated embryos were more sensitive to heavy metal exposure. From the germination results we can infer that the initial discharges of heavy metals into EFPC were lethal to certain species in the seed bank. We observed from our germination study that each species tested reacted differently to the metal compound and concentration applied. Knowledge about toxicity limits for tree species is important in understanding species succession following a disturbance.

The toxic effects of mercury depend on its chemical form and concentration, with methyl mercury being the most toxic. Mercury along with other heavy metal pollutants have been shown to be deleterious to aquatic plants and animals, soil microbial, plant and fungal communities. In ecosystems exposed to heavy metals, mycorrhizae have been shown to be effective in increasing heavy metal tolerance of their host plant. But the composition of the forest and tree/mycorrhizae interaction may or may not be affected directly by acute/chronic toxicity.

Acknowledgements

Financial support for this research was provided by The Department of Forestry Wildlife and Fisheries at the University of Tennessee, Knoxville (UTK) and Southern Regional Education Board. Thanks to the Department of Energy (DOE) Oak Ridge, TN for access

to sampling sites. Special thanks to Jeremie Damay who conducted all the germination studies and helped with soil sampling.

References

Agerer, R., 1991. Characterization of ectomycorrhiza. *In* Norris JR, Read DJ, Varma AK (eds). Techniques for the study of mycorrhiza. Methods Microbiology. 23, 25-73.

Auge, R. M., Moore, J. L., 2005. Arbuscular mycorrhizal symbiosus and plant drought resistance. *In*: Mehrotra VS, editor. Mycorrhiza: role and applications. New Dehli, Allied Publishers Limited, pp. 136-162.

Biester, H., Muller, G., Scholer, H.F., 2002. Binding and mobility of mercury in soil contaminated by emissions form chlor-alkali plant. The Science of The Total Environment. 284, 191-203.

Boening, D. W., 2000. Ecological effects, transports, and fate of mercury: a general review. Chemosphere. 40, 1335-1351.

Bontidean, I., Mortari, A., Leth, S., Brown, N. L., Karlson, U., Larsen, M. M., 2004. Biosenors for detection of mercury in contaminated soils. *Environmental Pollution*, 131, 255-262.

Bonner, F., Karrfalt, R. P., 2008. The Woody Seed Manual. Agriculture Handbook No. 727. Washington, D.C. U.S. Department of Agriculture, Forest Service, pp 1223.

Burger, J., Campbell, K., Campbell, T., Shukla, T., Dixon, C., Gochfield, M., 2005. Use of Central Stonerollers (Cyprinidae: *Campostoma anomalum*) From Tennessee as a Bioindicator of Metal Contamination. Environmental Monitoring and Assessment. 110, 171-184.

Canham, C. D., 1989. Different responses to gaps among shade-tolerant tree species. Ecology. 70, 548-550.

Del Val, C., Barea, J. M., Azcon-Aguilar, C., 1999. Diversity of Arbuscular Mycorhhizal Fungus Populations in Heavy-Metal-Contaminated Soils. Applied and Environmental Micorbiology. 65, 718-723.

Fargasova, A., 1994. Effect of Pb, Cd, Hg, As and Cr on Germination and Root Growth of *Sinapis alba* Seeds. Bulletin Environmental Contamination Toxicology. 52, 452-456.

Gadd, G. M., Griffiths, A. J., 1978. Microorganisms and heavy metal toxicity. Microbial Ecology. 4, 303-317.

Gadd, G. M., 1993. Interaction of fungi with toxic metals. New Phytologist. 124, 25-60.

Gilmour, J. T., Miller, M. S., 1973. Fate of a Mercuric-Mercurous Chloride Fungicide

Added to Turfgrass. Journal of Environmental Quality. 2, 145-148.

Griffioen, W. A., Iestwart, J. H., Ernst, W. O., 1994. Mycorrhizal infection of *Agrostis capillaries* population on a copper-contaminated soil. Plant and Soil. 158 (1), 83-89.

Han, F. X., Su, Y. M., David, L., Waggoner, Charles A., Plodinec, M. J., 2006. Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. The Science of the Total Environment. 368, 753-768.

Haselwandter, K., Dowen, G. D., 1996. Mycorrhizal relations in trees for agroforestry and land rehabilitation. Forestry Ecology Management. 81, 1–17.

Helmisaari, H. S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M., Vanha-Majamaa, I., 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air, and Soil Pollution. 85, 1727-32.

Kelsh, D. J., Parsons, M.W., 1997. Department of Energy Sites Suitable for Electrokinetic Remediation. Journal of Hazardous Material. 55, 109-116.

Kim, Y., Yang, Y., Lee, Y., 2002. Pd and Cd uptake in rice roots. Physiologia Plantarum. 116, 368-372.

Krugman, S. L., Jenkinson, J. L., 2008. The woody seed manual. Bonner, F. T., Karrfalt, (eds.) US Forest Service, Agriculture Handbook no. 727. Washington D.C. pp. 809-847.

Li, W., Khan, M. A., Yamaguchi, S., Kamiva, Y., 2005. Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. Plant Growth Regulation. 46, 45-50.

Lindberg, S. E., Jackson, D. R., Huckabee, S. A. Janzen, J. A., Levin, M. J., Lund, J. R., 1979. Atmospheric emission and plant uptake of mercury from agricultural soils near the Almaden mercury mine. Journal of Environmental Quality. 8, 572-578.

Liang, Y., Guo, L. D., Du, X. J., Ma, K. P., 2007. Spatial structure and diversity of woody plants and ectomycorrhizal fungus sporocarps in a natural subtropical forest. Mycorrhiza. 17, 271-278.

Lodenius, M., 1994. Mercury in Terrestrial Ecosystems: A Review. Mercury Pollution. Boca Raton, CRC Press.

Miller, M. R., Jastrow, J. D., 1992. The application of VA mycorrhizae to ecosystem restoration and reclamation. In M. J. Allen (Eds.), Mycorrhhizal functioning. Chapman and Hall, New York, pp. 488–517.

Marschner, H., 1995. Mineral nutrition of higher plants. 2nd eds., London, San Diego, Academic Press, pp. 596-657.

Munzuroglu, O., Geckil, H., 2002. Effects of metals on seed germination, root elongation, and coleoptiles and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus*. Archives of Environmental Contamination and Toxicology. 43, 203-213.

Nadkarni, R. A., 1984. Applications of microwave oven sample dissolution in analysis. Analytical Chemistry. 56, 2233-2237.

Nriagu, J. O., Pacyna, J. M., 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature. 333, 134-139.

Revis, N. W., Osborne, T. R., Holdsworth, G., Hadden C., 1989. Distribution of Mercury Species in Soil from a Mercury-Contaminated Site. Water, Air, and Soil Pollution. 45, 105-113.

SAIC, Science Application International Corporation, 1994. East Fork Poplar Creek-Sewer Line Beltway Remedial Investigation Report: Addendum. Oak Ridge, TN, U.S. Department of Energy.

Salemaa, M., Vanha-Majamaa, I., Derome, J., 2001. Understory vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution. 112, 339-350.

Schroeder, W. H., Munthe, J., 1998. Atmospheric Mercury- An Overview. Atmospheric Environment. 32, 809-822.

Schuster, E., 1991. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes- a review of the literature. Water, Air, and Soil Pollution. 56, 667-680.

Shi, L., Guttenberger, M., Kottke, I., Hampp, R., 2002. The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi. Mycorrhiza. 12 (6), 303-311.

Stewart, A. J., Haynes, G. J. Martinez, M. I., 1992. Fate and biological effects of contaminated vegetation in Tennessee stream. Environmental Toxicology and Chemistry. 11, 653-664.

Tsai, Y., Olson, B., 1990. Effects of Hg^{2+}, CH_3-Hg^{+}, and Temperature on the Expression of Mercury Resistance Genes in Environmental Bacteria. Applied and Environmental Microbiology. 56, 3266-3272.

USEPA, United Stated Environmental Protection Agency, 1989. National priorities listed for uncontrolled hazardous waste sites. Federal Register. 54(223), 48184-48189.

Urcelay, C., Robledo, G., 2009. Positively relationship between wood size and basidiocarp production of polypore fungi in *Alnus acuminate* forest. Fungal Ecology. 2, 135-139.

Wang, B., Qui, Y.-L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16, 299-363.

Weissenhorn, I., Leyval, C., 1994. Differential tolerance to Cd and Zn of arbuscular mycorrhizal fungal spores isolated from heavy metal-polluted and unpolluted soils. Plant Soil. 167, 189-196.

Zasada, J. C., Strong, T. F., 2008. *Acer* L., maple. Pages 204-216, *In* The woody seed manual. Bonner, F. T., Karrfalt, (eds.) US Forest Service, Agriculture Handbook no. 727. Washington D.C. pp. 809-847.

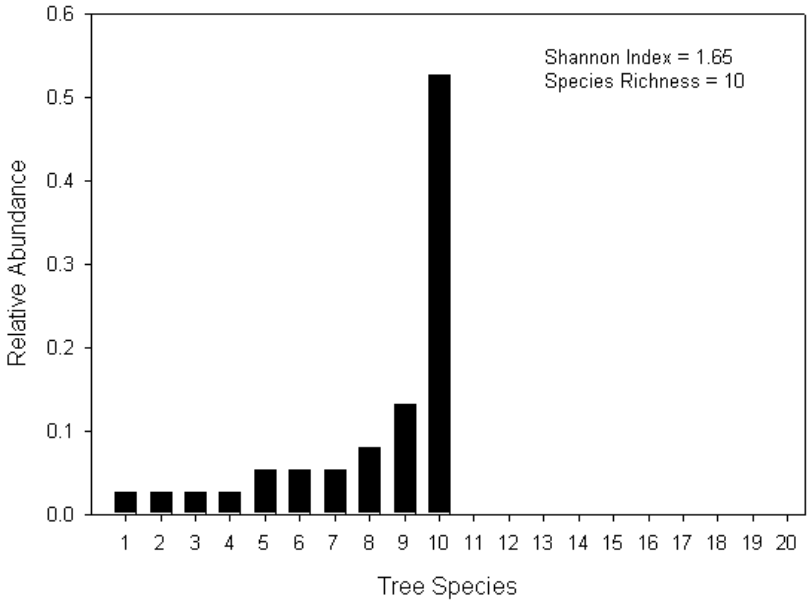
Appendix

Table 1. Occurrence of species within the 17 plots sampled along East Fork Poplar Creek, Anderson and Roane Co., TN

Species	Species #	B1PA	B1PB	B1PD	B1PE	B1PF	B1PG	B1PH	B2PA	B2PB	B2PC	B3PA	B3PB	B3PC	B3PD	B3PE	B3PF	B3PG
Platanus occidentalis	*1*	1	0	1	0	3	4	0	2	0	0	4	0	0	3	0	0	2
Oxydendrum arboreum	*2*	20	4	0	0	7	2	20	0	0	1	0	0	0	0	0	0	0
Carpinus caroliniana	*3*	2	1	0	0	0	0	0	0	0	0	0	0	0	2	0	9	0
Acer saccharum	*4*	3	8	0	6	8	6	0	0	0	0	0	0	0	0	0	0	0
Acer rubrum	*5*	5	8	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Quercus montana	*6*	2	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fraxinus americana	*7*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liquidambar styraciflua	*8*	2	1	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0
Liriodendron tulipifera	*9*	1	8	2	2	2	3	1	3	3	0	4	0	5	0	0	0	0
Aesculus flava	*10*	1	0	6	1	0	2	1	0	0	0	0	0	0	0	0	0	0
Ulmus rubra	*11*	0	0	0	0	1	2	1	0	0	0	2	0	0	0	0	0	0
Celtis occidentalis	*12*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fraxinus pennsylvanica	*13*	0	0	0	0	6	0	0	0	0	0	0	1	0	4	0	1	1
Acer negundo	*14*	0	0	0	1	0	3	1	8	3	13	2	0	0	0	0	0	0
Prunus serotina	*15*	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Lindera benzoin	*16*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Juniperus virginiana	*17*	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	4	0
Quercus phellos	*18*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Cornus flordia	*19*																	
Pine sp.	*20*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Unknown	*21*	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	4	2
Total		38	31	15	10	28	23	29	13	6	15	13	7	6	9	1	18	6

Fig 1. The relative abundance of tree species within each plot sampled along East Fork Poplar Creek (EFPC), Anderson and Roane, Tennessee. There were a total of 20 different tree species observed within the experimental area. Unknown species were grouped separately. Reference table 1 for tree species name with corresponding number.

Block 1 Plot A



Block 1 Plot B

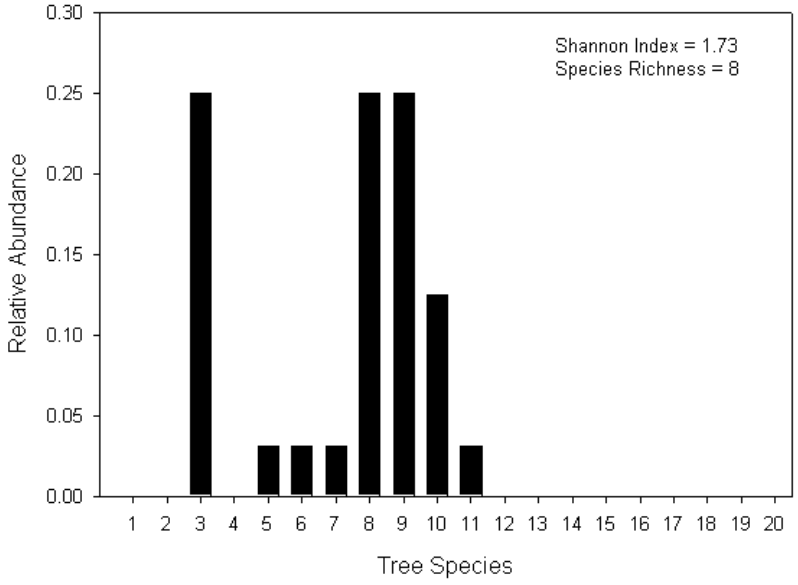
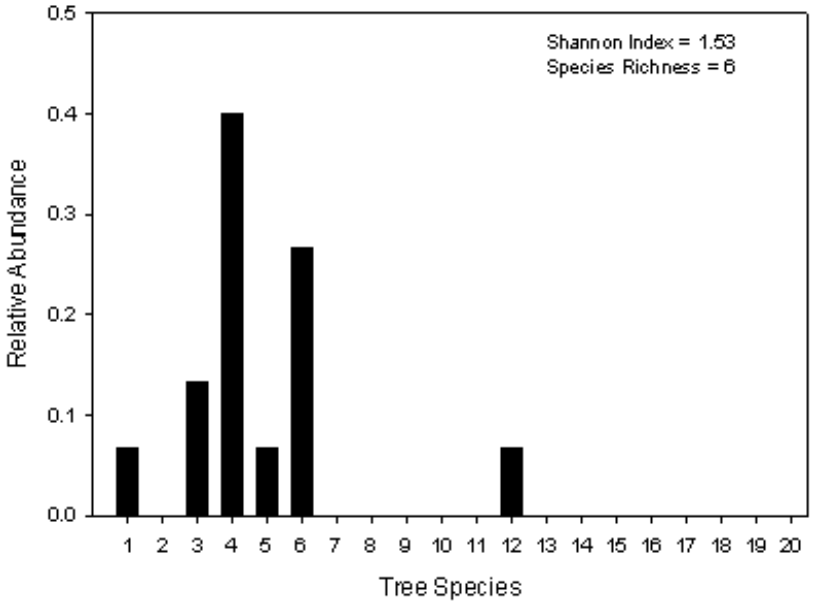


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 1 Plot D



Block 1 Plot E

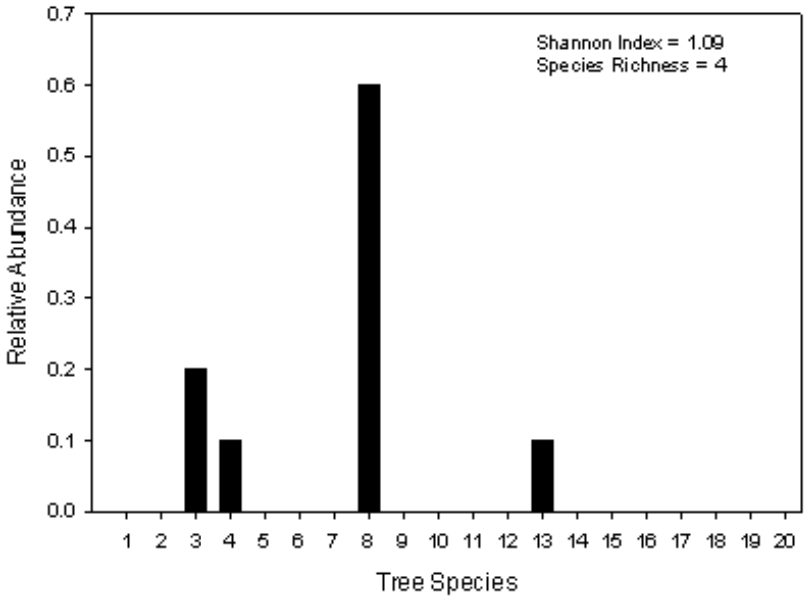
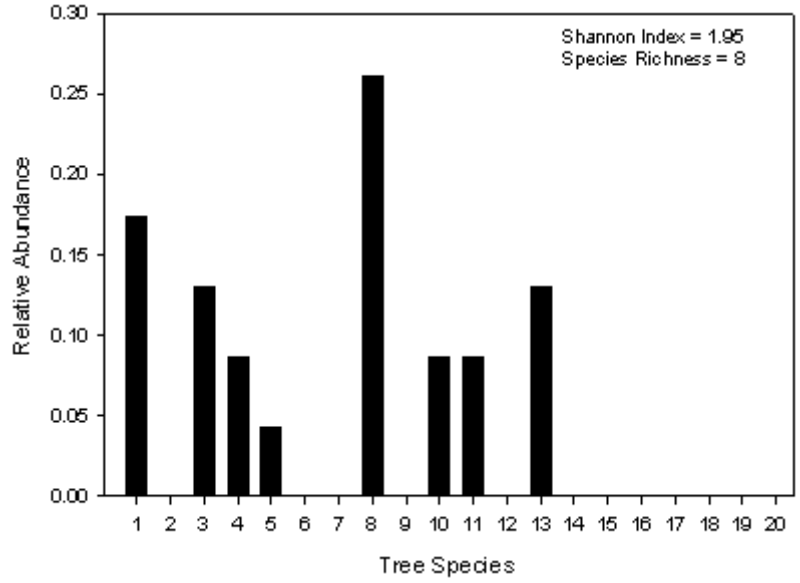


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 1 Plot F



Block 1 Plot G

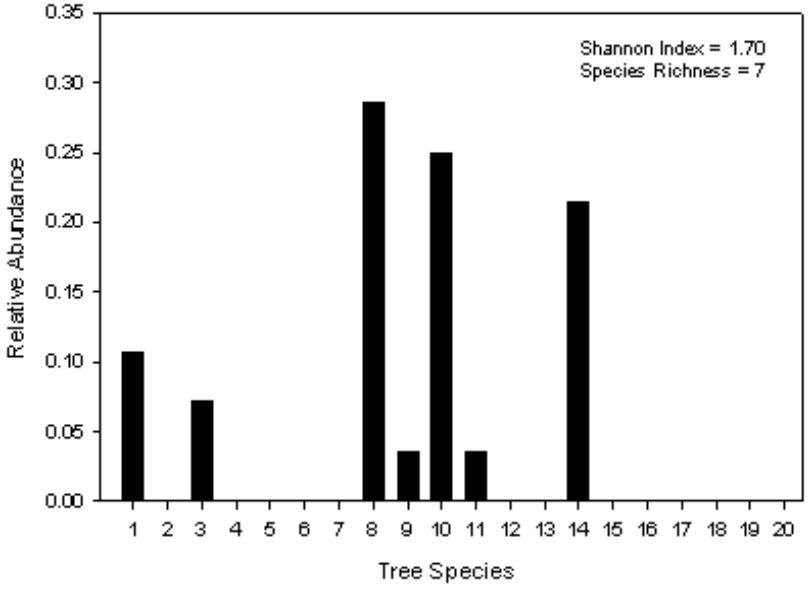
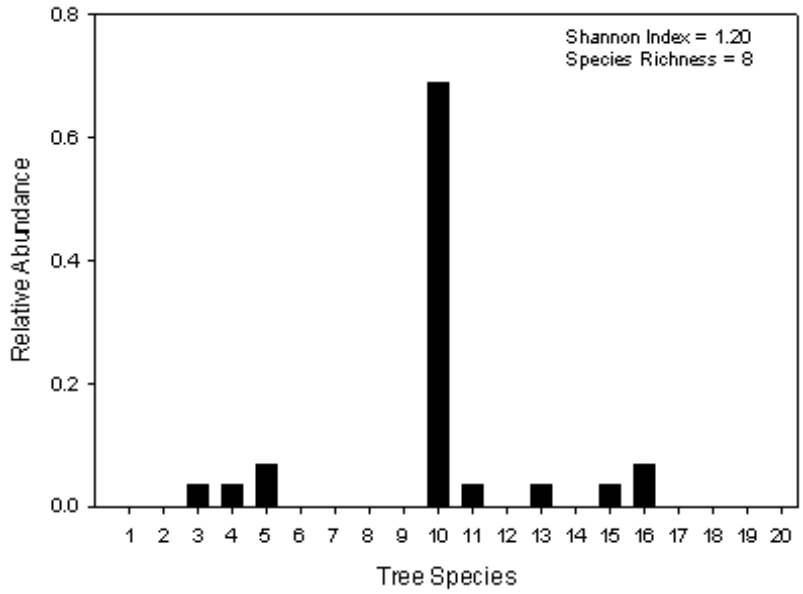


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 1 Plot H



Block 2 Plot A

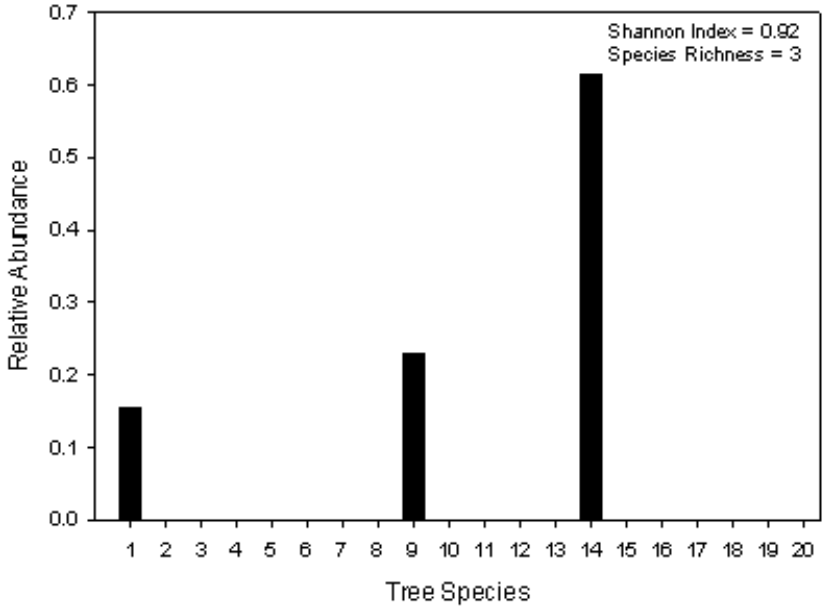
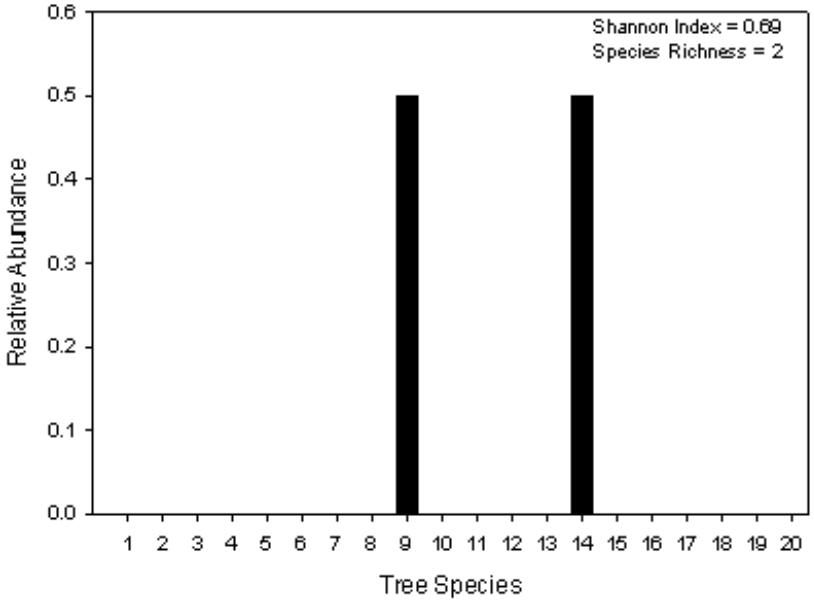


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 2 Plot B



Block 2 Plot C

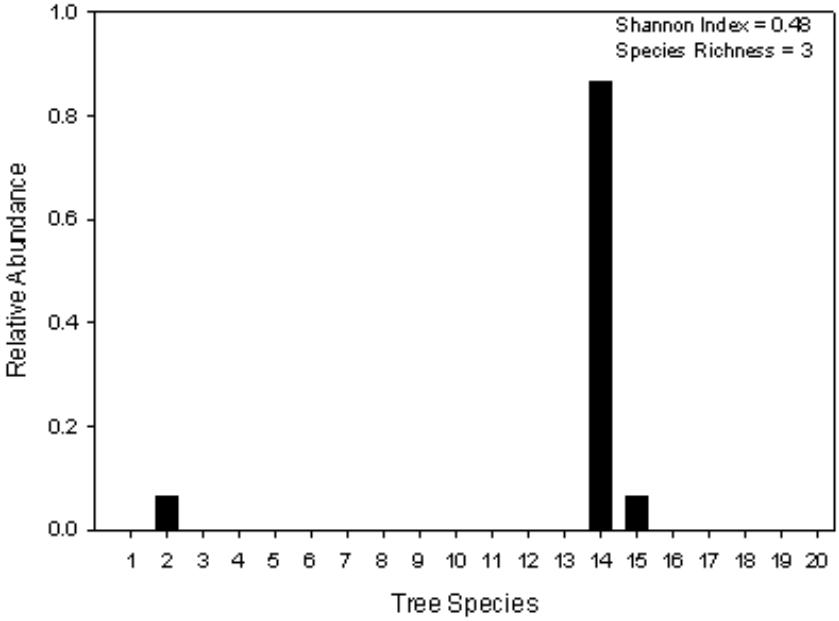
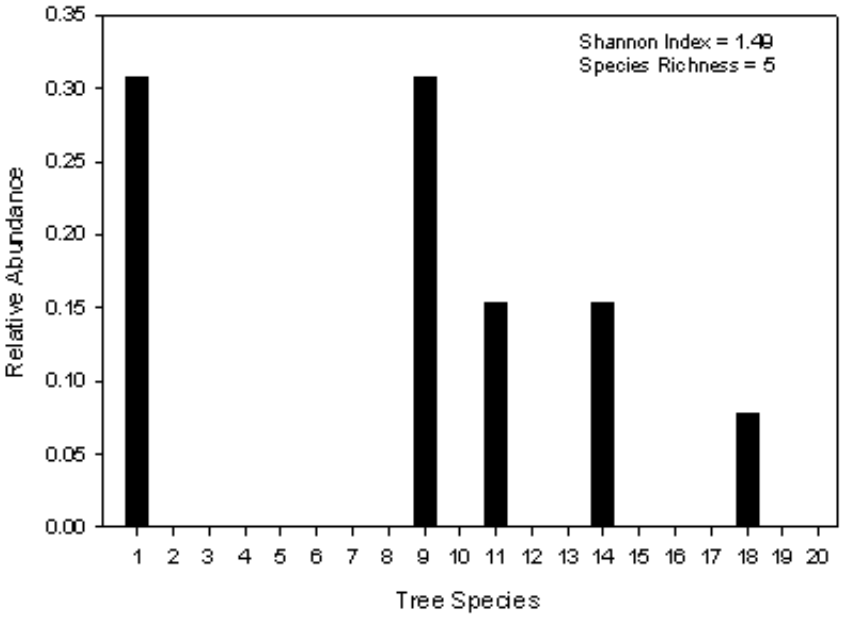


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 3 Plot A



Block 3 Plot B

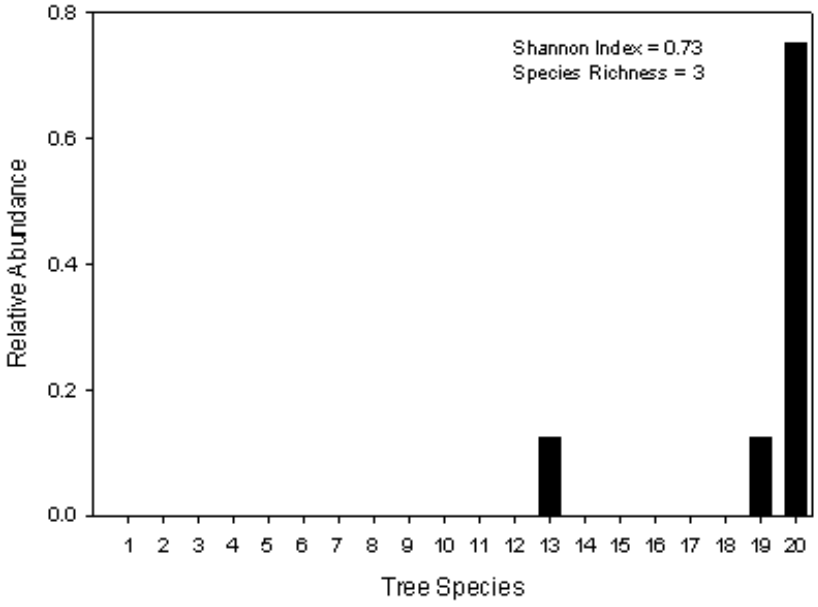
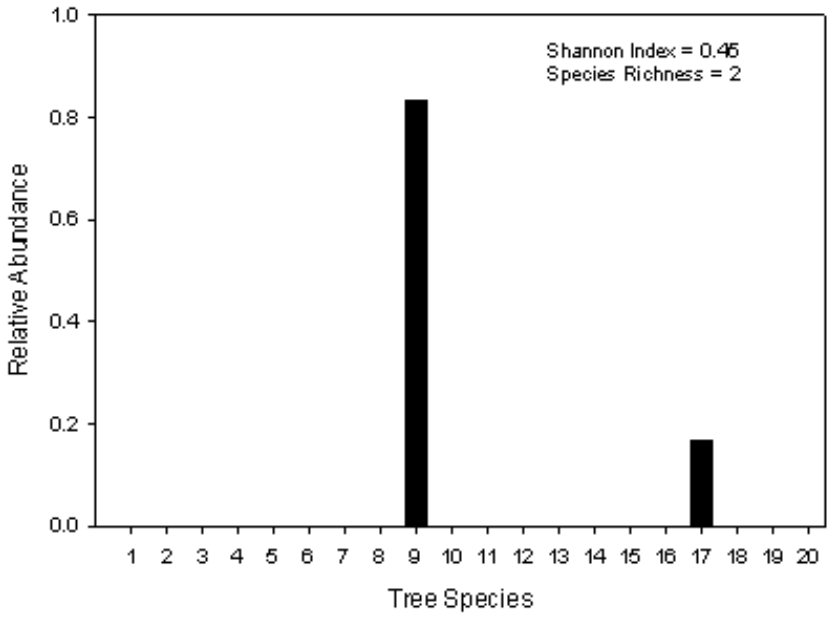


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 3 Plot C



Block 3 Plot D

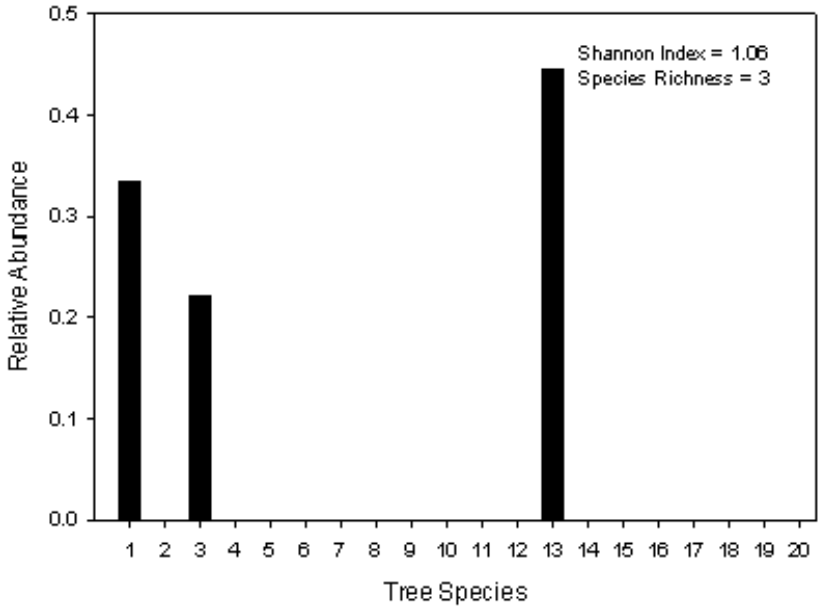
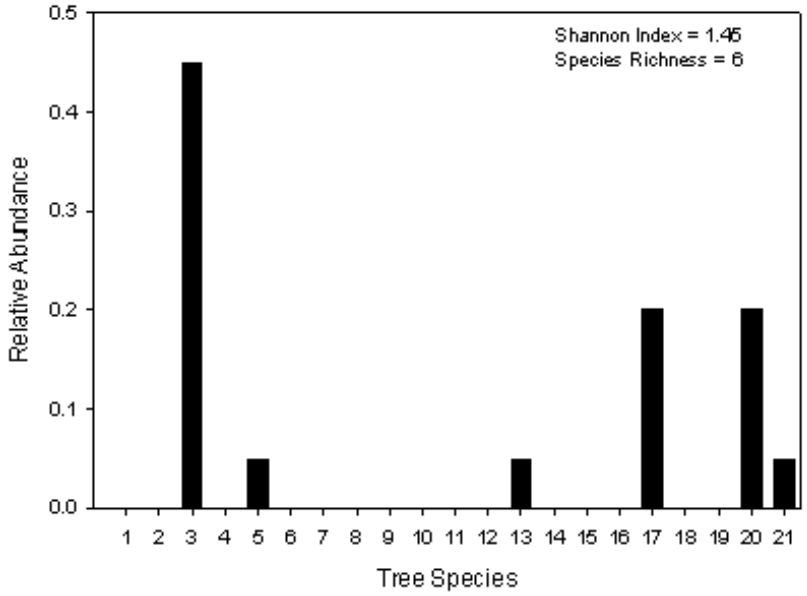
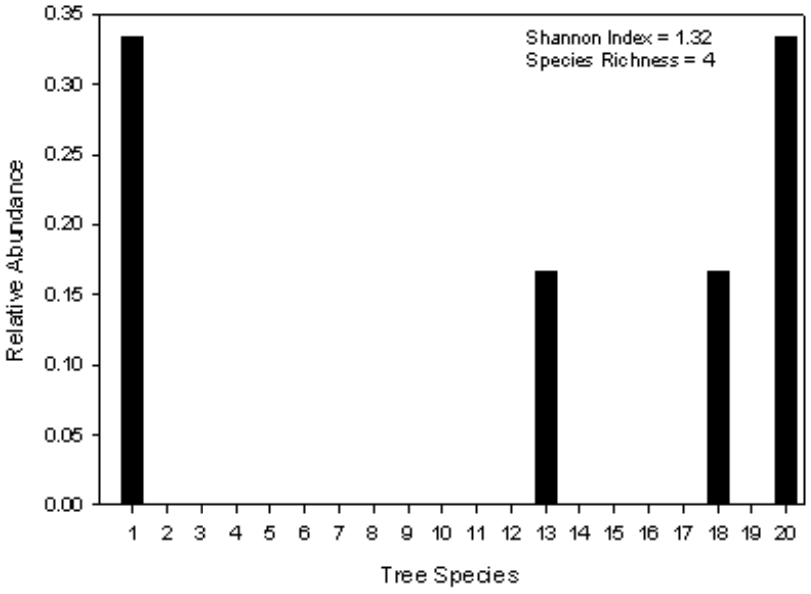


Fig. 1 continued. Relative abundances of tree species within block/plots along EFPC.

Block 3 Plot F



Block 3 Plot G



Protocol: NH_4OAc Exchangeable Bases and CEC by Automatic Extractor

Reagents:

1. Ammonium Acetate (1 N NH_4OAc, pH 7.0). Dissolve 38.5 g of NH_4OAc in deionized water in a 1 liter volumetric flask. Add 14.2 ml of glacial acetic acid and bring to 1000ml with deionized water.
2. Ethanol 95%
3. Acidified Sodium Solution. Dissolve 100 g NaCl in 500ml of deionized water, add 5 ml of 1 N HCl bring to 1000 ml with deionized water.

Equipment:

Automatic extractor with syringes, reservoir tubes, and filter pulp.

Procedure:

1. Tightly pack approximately 1 g of filter pulp into the bottom of individual syringe barrels. Weigh 2.5 g soil sample per syringe barrel.
2. Connect sample syringes to extracting syringes on the automatic extractor. Add 10 ml of Ammonium Acetate using 10 ml Oxford pipet to wash sample syringe; allow to stand for 20 min. With the extractor set to 30-minute rate, extract until solution is 1 cm above sample. Connect reservoir tubes to sample syringes; add 40 ml Ammonium Acetate to each reservoir tube using 20 ml repipet. Set extractor to the 12 hour-extraction rate and extractor overnight. Volume of extract recovered should be 50 ml $\pm$ and retain extract for determination of exchangeable bases. Put solution into a 50 ml falcon tube.

3. Connect sample syringes to extracting syringes on the automatic extractor. Wash down the sides with 10 ml ethanol from a repipet, allow to stand for 20 minutes. With extractor set to 30-min rate, extract until extractant is 1 cm above sample. Connect reservoir tubes to sample syringe; add 40 ml ethanol to each reservoir tube. Set extractor to the 2-hour rate. Extract until no ethanol remains on the sample. Disconnect the bottom syringe and squirt the alcohol wash down the sink. Reconnect the lower syringe. Reconnect the reservoir syringe and add 40 ml alcohol and extract a second time omitting sample stirring. The second extraction should be carried out until there is no alcohol left in the sample tube.
4. Displacement and Distillation. Connect sample syringes to extracting syringes on the automatic extractor. Add 10 ml Acidified Sodium Solution, using the 10 ml Oxford, to wash sample syringe; allow to stand for 20 min. With extractor set to 30-min rate, extract until extractant is 1 cm above sample. Connect reservoir tubes to sample syringes; add 40 ml Acidified Sodium Solution to each reservoir tube. Set extractor to the 2-hour extraction rate and extract. Volume of extract recovered should be 50 ml $\pm$ 1 ml. Put solution into a 50 ml falcon tube.

Protocol: Distillation for Use of Rapid-Still: For CEC Ammonium Distillation

Reagents: 2 N NaOH [80g NaOH per liter of solution]

0.01 N standard acid [HCl or H_2SO_4]

80 g boric acid and 80 ml mixed indicator solution per 4 liters

To make the mixed indicator solution:

Combine 0.099 grams bromocresol green and 0.066 g methyl red in an agate; grind them gently in ethanol. Quantitatively transfer this to a 100 ml volulmetric flask and bring to volume with 95% ethanol. Allow to stand overnight. Before bringing the indicator mix to final volume, adjust solution pH to 5 with a small amount of 0.1 N NaOH.

Procedure:

Preparation of equipment: Add 500 ml of distilled water to an 800 ml Kjeldahl flask. Make sure to add boiling chips. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

Distillation: Forty milliters of sample, 10 ml of 0.1 NaOH and 10 ml of boric acid indicator solution to a 800 ml Kjeldahl flask. Distill 35 ml at 1 ml/min into a 50 ml beaker.

Tritrimetric determination: Add 3 drops of mixed indicator to the distillate and titrate the ammonia with 0.02 N H_2SO_4 until solution turns pink. Match the end point against a blank containing the same volume of distilled water and H_3BO_3 solution. Cation exchange capacity was determined using the following equation:

CEC (in meq/ 100 g soil) = V x 0.02 N x 40 ml/ 10 ml x 100 g/Ms

Where,

V= Volume of 0.02 H_2SO_4 spent for titration, in ml

40 ml = Total volume of 20 5 NaCl, used to substitute the NH^{4+}

10 ml = Volume of filtrate used for distillation

Ms= Weight of soil used per sample.

Table 2. Test for Fixed Effects for

Platanus occidentalis

Effect	Num DF	Den DF	F Value	Pr > F
MER	1	6	0.43	0.5375
CON	5	30	49.01	<.0001
MER*CON	5	30	2.84	0.0322
DAY	4	144	250.71	<.0001
MER*DAY	4	144	1.58	0.1840
CON*DAY	20	144	24.79	<.0001
MER*CON*DAY	20	144	2.19	0.0042

Pinus echinata

Effect	Num DF	Den DF	F Value	Pr > F
MER	1	3	11.91	0.0409
CON	5	30	17.33	<.0001
MER*CON	5	30	16.60	<.0001
DAY	4	144	257.36	<.0001
MER*DAY	4	144	3.19	0.0153
CON*DAY	20	144	6.23	<.0001
MER*CON*DAY	20	144	6.87	<.0001

Pinus taeda

Effect	Num DF	Den DF	F Value	Pr > F
MER	1	3	5.39	0.1030
CON	5	30	32.31	<.0001
MER*CON	5	30	7.64	<.0001
DAY	4	144	304.48	<.0001
MER*DAY	4	144	9.51	<.0001
CON*DAY	20	144	13.59	<.0001
MER*CON*DAY	20	144	8.41	<.0001

Platanus occidentalis

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
66	ch	0	7	18.2500	1.1339	A
67	ch	0	12	19.2500	1.1339	A
68	ch	0	22	0.5000	1.1339	FG
69	ch	0	37	0.2500	1.1339	FG
70	ch	0	44	0.2500	1.1339	FG
71	ch	5	7	17.5000	1.1339	A
72	ch	5	12	19.0000	1.1339	A

73	ch	5	22	6.7500	1.1339	C
74	ch	5	37	4.5000	1.1339	CD
75	ch	5	44	4.2500	1.1339	CDE
76	ch	10	7	10.5000	1.1339	B
77	ch	10	12	11.0000	1.1339	B
78	ch	10	22	1.0000	1.1339	FG
79	ch	10	37	0.5000	1.1339	FG
80	ch	10	44	0.5000	1.1339	FG
81	ch	50	7	1.7500	1.1339	DEFG
82	ch	50	12	3.0000	1.1339	DEF
83	ch	50	22	-151E-17	1.1339	G
84	ch	50	37	-134E-17	1.1339	G
85	ch	50	44	6.58E-15	1.1339	G
86	ch	100	7	1.7500	1.1339	DEFG
87	ch	100	12	2.2500	1.1339	DEFG
88	ch	100	22	3.45E-16	1.1339	FG
89	ch	100	37	2.46E-16	1.1339	FG
90	ch	100	44	9.21E-15	1.1339	FG
91	ch	500	7	1.2500	1.1339	EFG
92	ch	500	12	1.0000	1.1339	FG
93	ch	500	22	1.44E-14	1.1339	FG
94	ch	500	37	1.68E-14	1.1339	FG
95	ch	500	44	9.16E-16	1.1339	FG

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
96	hg	0	7	14.5000	1.1339	AB
97	hg	0	12	13.5000	1.1339	B
98	hg	0	22	1.7500	1.1339	DE
99	hg	0	37	1.7500	1.1339	DE
100	hg	0	44	1.7500	1.1339	DE
101	hg	5	7	17.0000	1.1339	A
102	hg	5	12	16.0000	1.1339	AB
103	hg	5	22	2.7500	1.1339	DE
104	hg	5	37	3.0000	1.1339	DE
105	hg	5	44	3.0000	1.1339	DE
106	hg	10	7	13.0000	1.1339	B
107	hg	10	12	14.5000	1.1339	AB
108	hg	10	22	4.2500	1.1339	CD
109	hg	10	37	4.0000	1.1339	CD
110	hg	10	44	4.0000	1.1339	CD
111	hg	50	7	6.5000	1.1339	C
112	hg	50	12	1.2500	1.1339	DE
113	hg	50	22	-287E-17	1.1339	EF
114	hg	50	37	-34E-16	1.1339	EF
115	hg	50	44	4E-15	1.1339	EF
116	hg	100	7	3.5000	1.1339	CD
117	hg	100	12	3.5000	1.1339	CD
118	hg	100	22	-325E-17	1.1339	EF
119	hg	100	37	-384E-17	1.1339	EF
120	hg	100	44	8.33E-17	1.1339	EF
121	hg	500	7	2.7500	1.1339	DF
122	hg	500	12	2.5000	1.1339	DE
123	hg	500	22	4.32E-15	1.1339	E
124	hg	500	37	-784E-18	1.1339	E

125	hg	500	44	-183E-16	1.1339	E

Pinus echinata

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
66	ch	0	7	8.2500	1.4253	D
67	ch	0	12	20.2500	1.4253	AB
68	ch	0	22	15.5000	1.4253	C
69	ch	0	37	14.0000	1.4253	C
70	ch	0	44	14.0000	1.4253	C
71	ch	5	7	7.7500	1.4253	D
72	ch	5	12	21.2500	1.4253	A
73	ch	5	22	16.2500	1.4253	C
74	ch	5	37	15.7500	1.4253	C
75	ch	5	44	15.5000	1.4253	C
76	ch	10	7	6.0000	1.4253	DEF
77	ch	10	12	20.5000	1.4253	AB
78	ch	10	22	14.7500	1.4253	C
79	ch	10	37	14.2500	1.4253	C
80	ch	10	44	14.2500	1.4253	C
81	ch	50	7	3.5000	1.4253	EFG
82	ch	50	12	17.2500	1.4253	BC
83	ch	50	22	7.5000	1.4253	D
84	ch	50	37	4.7500	1.4253	DEF
85	ch	50	44	1.7500	1.4253	G
86	ch	100	7	7.0000	1.4253	DE
87	ch	100	12	21.5000	1.4253	A
88	ch	100	22	2.7500	1.4253	FG
89	ch	100	37	0.7500	1.4253	G
90	ch	100	44	0.2500	1.4253	G
91	ch	500	7	-16E-15	1.4253	G
92	ch	500	12	2.7500	1.4253	FG
93	ch	500	22	-333E-16	1.4253	G
94	ch	500	37	-283E-16	1.4253	G
95	ch	500	44	-782E-16	1.4253	G

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
96	hg	0	7	7.5000	1.4253	EFGH
97	hg	0	12	21.7500	1.4253	A
98	hg	0	22	11.7500	1.4253	BCD
99	hg	0	37	10.0000	1.4253	BCDE
100	hg	0	44	10.0000	1.4253	BCDE
101	hg	5	7	7.2500	1.4253	EFGH
102	hg	5	12	19.7500	1.4253	A
103	hg	5	22	8.7500	1.4253	DEFG
104	hg	5	37	8.0000	1.4253	DEFGH
105	hg	5	44	7.7500	1.4253	EFGH
106	hg	10	7	5.5000	1.4253	FGH
107	hg	10	12	19.7500	1.4253	A

108	hg	10	22	13.2500	1.4253	B
109	hg	10	37	13.2500	1.4253	B
110	hg	10	44	13.0000	1.4253	BC
111	hg	50	7	5.2500	1.4253	GH
112	hg	50	12	20.5000	1.4253	A
113	hg	50	22	13.0000	1.4253	BC
114	hg	50	37	12.7500	1.4253	BC
115	hg	50	44	13.0000	1.4253	BC
116	hg	100	7	4.5000	1.4253	H
117	hg	100	12	20.5000	1.4253	A
118	hg	100	22	10.7500	1.4253	BCDE
119	hg	100	37	9.2500	1.4253	CDEF
120	hg	100	44	8.7500	1.4253	DEFG
121	hg	500	7	4.5000	1.4253	H
122	hg	500	12	19.2500	1.4253	A
123	hg	500	22	11.7500	1.4253	BCD
124	hg	500	37	11.0000	1.4253	BCDE
125	hg	500	44	10.7500	1.4253	BCDE

Pinus taeda

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
66	ch	0	7	0.7500	1.4102	FGHI
67	ch	0	12	15.0000	1.4102	ABC
68	ch	0	22	16.0000	1.4102	AB
69	ch	0	37	16.0000	1.4102	AB
70	ch	0	44	15.7500	1.4102	AB
71	ch	5	7	1.7500	1.4102	FGHI
72	ch	5	12	15.5000	1.4102	AB
73	ch	5	22	15.2500	1.4102	ABC
74	ch	5	37	15.5000	1.4102	AB
75	ch	5	44	15.7500	1.4102	AB
76	ch	10	7	2.2500	1.4102	FGHI
77	ch	10	12	12.7500	1.4102	BD
78	ch	10	22	15.2500	1.4102	AC
79	ch	10	37	13.7500	1.4102	ABC
80	ch	10	44	14.0000	1.4102	ABC
81	ch	50	7	1.2500	1.4102	HI
82	ch	50	12	11.7500	1.4102	CD
83	ch	50	22	4.2500	1.4102	EF
84	ch	50	37	3.7500	1.4102	FG
85	ch	50	44	2.7500	1.4102	FGHI
86	ch	100	7	0.5000	1.4102	GHI
87	ch	100	12	7.5000	1.4102	E
88	ch	100	22	1.2500	1.4102	FGHI
89	ch	100	37	0.2500	1.4102	GHI
90	ch	100	44	0.2500	1.4102	GHI
91	ch	500	7	0.7500	1.4102	FGHI

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
92	ch	500	12	2.5000	1.4102	FGH
93	ch	500	22	-468E-16	1.4102	I
94	ch	500	37	-326E-16	1.4102	I
95	ch	500	44	-986E-16	1.4102	I

---------- Effect=MER*CON*DAY Method=LSD(P<.05) ----------

Obs	MER	CON	DAY	Estimate	Standard Error	Letter Group
96	hg	0	7	2.7500	1.4102	FG
97	hg	0	12	17.0000	1.4102	A
98	hg	0	22	15.5000	1.4102	AB
99	hg	0	37	15.0000	1.4102	ABC
100	hg	0	44	14.7500	1.4102	ABC
101	hg	5	7	1.5000	1.4102	FG
102	hg	5	12	13.0000	1.4102	BCD
103	hg	5	22	10.7500	1.4102	DE
104	hg	5	37	10.2500	1.4102	E
105	hg	5	44	10.2500	1.4102	E
106	hg	10	7	1.7500	1.4102	FG
107	hg	10	12	15.7500	1.4102	AB
108	hg	10	22	15.5000	1.4102	AB
109	hg	10	37	14.0000	1.4102	ABCD
110	hg	10	44	14.7500	1.4102	ABC
111	hg	50	7	1.2500	1.4102	FG
112	hg	50	12	13.2500	1.4102	BCDE
113	hg	50	22	13.7500	1.4102	ABCDE
114	hg	50	37	13.7500	1.4102	ABCDE
115	hg	50	44	14.0000	1.4102	ABCD
116	hg	100	7	0.5000	1.4102	G
117	hg	100	12	10.5000	1.4102	DE
118	hg	100	22	11.5000	1.4102	CDE
119	hg	100	37	11.5000	1.4102	CDE
120	hg	100	44	11.5000	1.4102	CDE
121	hg	500	7	0.5000	1.4102	G
122	hg	500	12	4.2500	1.4102	F
123	hg	500	22	4.2500	1.4102	F
124	hg	500	37	4.5000	1.4102	F
125	hg	500	44	4.2500	1.4102	F

PART III
THE EFFECT OF MYCORRHIZAL FUNGI ON MERCURY UPTAKE BY SYCAMORE SEEDLINGS (PLATANUS OCCIDENTALIS)

Abstract

A greenhouse study was conducted to assess the phytotoxic effects of different mercuric solutions on *Platanus occidentalis* (American Sycamore) seedlings; and investigate enhanced tolerance of sycamore seedlings grown in mercury contaminated soil through mycorrhizal fungi association. We also measured vegetation stress by Near Infrared (NIR) spectroscopy. Wavelengths were examined that are specific to chlorophyll and several carotenoids, which are involved in photosynthesis: 430 nm (Chl *a*), 448 nm (Chl *b*, carotenoids), 471 nm (carotenoids), 642 nm (Chl *b*), 662 & 680 nm (Chl *a*). Principal component analysis (PCA) was performed to identify patterns in sycamore leaf spectral data. Total mercury in roots at the end of the experimental period ranged from 4.9 to 3266 mg kg^{-1} Hg, whereas in shoot (stem and leaf) total mercury ranged from 1.0 to 242.2 mg kg^{-1} Hg. Both mercury treatment and inoculation treatment had no effect on mercury concentration in roots of sycamore seedlings. Mycorrhizal fungi were present on the roots of sycamore seedlings in all mercuric treatments. Significant changes occurred in levels of all pigments sampled (p430, p448, p471, p642, p662, and p680) over the course of the experiment. NIR spectroscopy was not sensitive enough to detect other chemical changes to foliage following mercury application.

Introduction

Globally, mercury (Hg) pollution has become an important environmental issue. Mercury occurs in the metallic form and as sulfide ores in nature. Mercury in the environment undergoes different transformations of its chemical form. Atmospheric movement of mercury is mainly from elemental vapors, methylmercury, or mercury bound to particulates (Morita et al., 1998). In aquatic and terrestrial environments, inorganic Hg is methylated to methyl-mercury species which are readily accumulated in aquatic and terrestrial animals (Burger et al., 2005; Campbell et al., 2005). Mercury in the environment can move into plants, which are therefore the main entry point into the food chain.

Mercury uptake in plant tissue may occur through oxidized forms of Hg (II) or methyl mercury absorbed onto the soil particles or dissolved in soil water entering through roots. Plant foliage may also uptake elemental mercury (Hg°) into stomata through volatilization of Hg-enriched soil. Several biochemical and physiological processes in higher plants are disrupted due to mercury exposure (Beauford et al., 1977; Godbold, 1991; Godbold & Hutterman, 1986; Patra et al., 2004; Schlegel et al., 1987). These include reduction in chlorophyll synthesis (Prasad and Prasad, 1987), water uptake

(Barcelo and Poschenrider, 1990; Beauford et al., 1977), photosynthesis, and transpiration rates (Schlegel et al., 1987).

Background

Plant and fungal mutualistic symbiosis

One important mutualistic symbiosis in the plant kingdom is that of mycorrhizae (fungus roots). Mycorrhizae are mutual beneficial symbiotic associations shared between fungi and roots. Fungal cell wall components have been shown to alter movement of solutes into plant roots. Components such as chitin, cellulose, and cellulose derivatives have been found to bind heavy metals (Galun et al., 1983). The fungal sheath can act as a barrier, preventing heavy metal transport to the root cortex of its host plant. Some fungi may exhibit a hydrophobic fungal apoplast, restricting apoplastic transport of water and ions (Unestam, 1991). Chelation of heavy metals by fungi has been linked to metal tolerance (Gadd, 1993). Several authors point out that mycorrhizal fungi exude organic acids (e.g. oxalic acid) and produce slime capable of binding metals within the fungal components (Lapeyrie et al., 1987; Denny and Ridge, 1995). Several fungal structures, or chemical substances released by fungi have been implicated in protecting plants from heavy metals in contaminated areas. The ability to restrict heavy metal entry into plant roots was shown to be due to the abundance of extramatrical mycelium (Galli et al., 1994).

Effects of mycorrhizal fungi on heavy metal uptake

Between 80 and 92% of surveyed land plant species and families are mycorrhizal, with endomycorrhizae being the predominate mycorrhiza (Wang and Qui, 2006). Enhanced mycorrhizal infection has been shown to be involved in heavy metal uptake and translocation of Cu, Zn, and Co in soil solution at low concentrations (Pacovsky, 1986; Rogers and Williams, 1986). However, Killham & Firestone (1983) have shown in bunchgrass (*Erhanta calycina*) inoculated with arbuscular mycorrhizal fungus (AMF) *Glomus fasciculatum*, growth in plant roots and shoots was reduced by increased uptake of Cu, Ni, Pb and Zn. Pine seedlings infected with the ectomycorrhizae fungus *Telephora terrestris* had increased Zn concentrations in needles when exposed to high levels of Zn pollution (Colpaert and Van Assche, 1992). On the other hand, mycorrhizal fungi have been shown to protect plants from heavy metals. Seedlings of *Pinus sylvestris* infected with the ectomycorrhizal fungus *Suillus bovines* had reduced Zn concentration in needles when exposed to high levels of Zn pollution (Colpaert and Van Assche, 1992). Pine seedlings infected with the ectomycorrhizae fungus *Paxillus involutus*, a species isolated from a heavy metal contaminated sites, were used to test the tolerance of those seedlings to increased cadmium (> 15 µM) stress. Non-mycorrhizal pine seedlings exhibited a 35% diminished biomass as compared to seedlings with mycorrhizae, which exhibited no biomass reduction (Schützendübel and Polle, 2002). Ectomycorrhizae accumulate metals within their fruit bodies and mycelium, with accumulation of metals being species dependent (Leyval, 1997). The ability to detect metal accumulation in endomycorrhizal fungi is difficult, to due to the need for a host plant for cultivation. Cooper and Tinker

(1978) have shown the accumulation and translocation of ^{65}Zn and ^{109}Cd from the soil solution to roots in the extraradical hyphae of endomycorrizae.

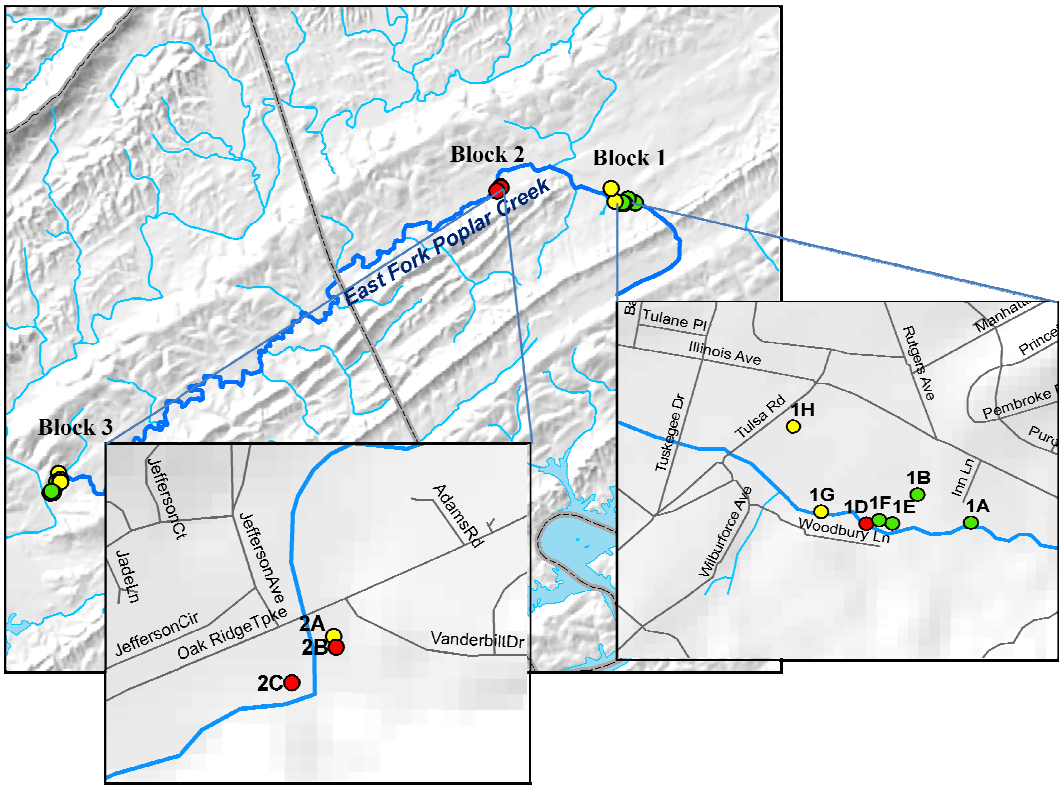
The purpose of this study was to investigate the tolerance of mycorrhizal association seedlings exposed to mercury. We test whether the inoculation treatment enhances tolerance to different mercuric applications. Using mercury contaminated soil known to have mycorrhizal fungi, American sycamore seedlings were inoculated with these soil samples and later grown in different mercuric solutions. American sycamore was selected because of its abundant presence in mercury contaminated soils along the creek bank of East Fork Poplar Creek in Oak Ridge, Tennessee. We also measured the leaves of American sycamore seedlings using Near Infrared Spectroscopy (NIR) as a tool to monitor chemical changes in these leaves from mercury exposure.

Methods

Greenhouse study

Historical soil mercury data obtained from Science Application International Corporation (SAIC, 1995) was used to establish plots with high (> 200 mg kg^{-1}) medium (50 -200 mg kg^{-1}) and low (< 50 mg kg^{-1}) levels of soil mercury along East Fork Poplar Creek (Figure 3.1). Soil cores obtained from plots recorded as “high”, were used to inoculate sycamore seed beds prior to germination. Eight seed beds, (two controls, two inoculated with soil cores from plot 1D, two inoculated with soil cores from plot 2A, and two inoculated with a combination of 1D and 2A (Combo)) were established with all beds containing sterilized 2:1 (by volume) vermiculite/sand media. Commercial sycamore seed with a Tennessee origin was obtained from Sheffield Seed Company, New

York. Seeds were sterilized for three minutes in a 10% bleach solution, soaked in deionized water for 1 hour and planted in the beds. This sterilization process was recommended by the company. Seed beds were placed in the greenhouse, allowed to establish for 2 months, and watered with 25% Hoalgand's solution throughout the study (Hoagland & Arnon, 1938).



*Mercury levels are denoted by color, red (high), yellow (medium), and green (low). Two plots, 1D and 2A were used to inoculate sycamore seedlings prior to the start of the mercury study.

Figure 3.1. Soil sampling sites along EFPC from Block 1 and Block 2.

After two months, sycamore seedlings were transplanted into individual pots containing sterilized 2:1 (by volume) vermiculite/sand media (400 g per pot) and allowed to establish for an additional 6 weeks at which point they were approximately 10 cm tall with several pairs of mature leaves.

Experimental Setup

A randomized complete block design was used. Seedlings established in the four inoculate treatments (1D, 2A, 1D + 2A, and no inoculate) were randomly assigned to tubs with a total of 24 seedlings per tub (6 seedlings for each inoculate treatment). Each tub represented one of the four mercury treatments (CH_3HgCl, $Hg(NO_3)_2$, $CH_3HgCl+Hg(NO_3)_2$, and no mercury). Tubs were used to keep contaminated solution contained (Table 3.1). Seedlings were watered once a week with either mercuric nitrate ($Hg(NO_3)_2$), methyl mercury chloride (CH_3HgCl), a combination of the two mercuric solutions or deionized water. Mercury solution was made such that total mercury concentration in a liter of deionized water was 100 mg kg^{-1}. Blocks were replicated once.

To minimize volatilization of mercuric compounds, which occurs rapidly above 30°C, a cooling system using chilled water circulating through copper tubing was set up below the tubs to keep soil temperature at or below 30° C during the experiment. Soil

temperature was taken three times a day (morning, afternoon and evening). The temperature during the month of September averaged 28.1°C during the day to 16.5°C at night and during the month of October averaged 22.4°C during the day to 9.2°C at night. Watering cans were used to water seedlings, directly watering the leaves of the seedlings. One liter of solution was applied to 24 seedlings randomly. Seedlings were treated

Table 3.1. Experimental setup: Randomized complete block design

[1]Tub1-$Hg(NO_{3)2}$	Tub 2- CH_3HgCl	Tub 3-$Hg(NO_3)_2$+ CH_3HgCl	Tub 4-Control
Control	1D	Control	Control
Combo	Control	1D	1D
2A	B2PA	2A	Combo
1D	Combo	Combo	2A
[2]Tub1-$Hg(NO_{3)2}$	Tub 2- CH_3HgCl	Tub 3-$Hg(NO_3)_2$+ CH_3HgCl	Tub 4-Control
Combo	Control	2A	1D
1D	1D	1D	2A
2A	2A	Control	Control
Control	Combo	Combo	Combo

Within a block (1or 2) there were four mercury and inoculate treatments.

weekly from 9/24/08 to 10/22/08. Daily observations were taken to note changes in foliar appearance such as color, curling and wilting. Any fallen leaves were collected and dried for later analysis.

NIR analysis of sycamore leaves

Spectra obtained from vegetation during stress or senescence typically show increased overall reflectance in the visible, due to loss of chlorophyll, and decreased reflectance in the NIR, due to damage to leaf cell walls and mesophyll tissue (Boyer et al., 1988; Carter, 1994; Smith et al., 2004). NIR spectra were collected from the leaves of living sycamore seedlings during the one month of mercury treatment using a portable leaf near infrared probe attached to a spectrometer (LabSpec® Pro). Eight spectra were

collected from sycamore seedlings leaves in each of the four mercury treatments on days 2, 4, 7, 10, 22 of the experiment. We measured vegetation stress by analyzing the reflectance spectra, therefore the entire 350-2500 nm spectral interval was examined. After initial analysis, we focused on the following wavelengths at which the maximum peaks of chlorophyll and several carotenoids are found: 430 nm (Chl *a*), 448 nm (Chl *b*, carotenoids), 471 nm (carotenoids), 642 nm (Chl *b*), 662 & 680 nm (Chl *a*) (Dunagan et al., 2007).

Pigment analyses

Repeated measures ANOVA was used to test the effects of each mercury treatment (CH_3HgCl, CH_3HgCl+Hg $(NO_3)_2$, Hg $(NO_3)_2$, control) on each of the six wavelengths characteristic of photosynthetic pigments, and collected from the visible region by near infrared spectroscopy (430 nm (Chl *a*), 448 nm (Chl *b*, caroteniods), 471 nm (caroteniods), 642 nm (Chl *b*), 662 & 680 nm (Chl *a*)). Before analysis, log transformation of pigment data was performed to normalize the data. Since there are 6 pigments x 4 treatments x 4 days (96 F-test), significance levels were adjusted to minimize Type 1 error using a Bonferroni adjustment where, 4 trt x 4 days = 16 tests, so 16 / .05 = 0.003 (Bland and Altman, 1995). The new alpha, $\alpha = 0.003$ was used instead of 0.05 to evaluate day test and paired days tests within each treatment. Day was a repeated measure. Statistical analyses were run in SAS 9.1.3.

Multivariate analyses of spectra

A multivariate technique is required to extract structural information from spectral data due to high inter-correlation between spectra. Principal component analysis (PCA) is

an unsupervised exploratory analysis of spectral data. Outliers can be detected and patterns in spectral data, through grouping or clustering, are observed. Multivariate analysis of the near-infrared spectra was performed using Unscrambler v. 9.2 software (CAMO Software Inc., Woodbridge, NJ). All spectra were modified by reduction (averaging) of wavelength by 4 and mean normalized prior to PCA analyses.

Mycorrhizal analysis

Sycamore seedlings were harvested by cutting the base of the stem at the root/shoot interface. Roots were quickly rinsed in a three step process (using deionized water) to remove vermiculite/sand material. Subsamples of roots were randomly selected and washed in deionized water and placed in cassettes, then were immersed in 10% KOH, boiled 3X for 20 minutes each to remove pigments, and rinsed 3 times in tap water and once in deionized water. Roots were then immersed in 3% hydrogen peroxide for 1 hour, rinsed 3-5 times in tap water and once in deionized water. Roots were acidified in 2% HCl for 1.5 hours and then immersed in 0.05% trypan blue strain for 1 hour. Roots were rinsed 2-3 times with distilled water and immersed in lactoglycerol solution (equal volume of 85% lactic acid, glycerol and distilled water) until analyzed. Additional sub-samples of roots were randomly selected and fixed in FAA (formalin-acetic acid-alcohol) to quantify ectomycorrhizal presence (Agué, 2005).

Roots were plated on slides and counted using a compound light microscope, starting at the bottom right end of each slide moving right to left; roots that came into view were assessed for presence or absence of vesicles or arbuscules (which indicates endomycorrhizal presence) until a hundred counts were obtained. Root length

colonization percentage was obtained by dividing the number of colonized by total number of roots. To assess ectomycorrhizae, FAA fixed roots were placed in a glass petri dish and counted using the grind line intersection method (Agerer, 1991).

Mercury Analysis

Sycamore seedling roots and shoots were harvested after one month of mercury treatment and analyzed for total mercury concentration. Fresh roots and shoots were rinsed in deionized water in a three step process to remove debris, and ground in liquid nitrogen. Samples were analyzed for total mercury concentration by cold vapor atomic absorption spectroscopy (CVAAS) by Western Kentucky University, Institute of Combustion Science and Environmental Technology.

Data Analysis

Analysis of variance (ANOVA) was run on original data to test for mean differences between blocks (1, 2), by inoculation treatments (1D, 2A, 1D +2A, and control) for each mercury treatment (CH_3HgCl, CH_3HgCl+Hg $(NO_3)_2$, Hg $(NO_3)_2$, control). The homogeneity of variance assumption was not met, so analyses were performed using a rank transformation. All data analyses used SAS 9.1.3 (SAS Inc., Cary, NC USA).

Results and Discussion

Total mercury concentrations in mercury treated roots ranged from 638.5 to 3438 mg kg^{-1} Hg, whereas in mercury treated shoots (stem and leaf) ranged from 78.6 to 246

mg kg^{-1} Hg. In sycamore seedling roots from the control treatment, total mercury ranged from 3 to 17 mg kg^{-1}, and in shoots ranged from 1.0 to 3.8 mg kg^{-1} Hg. These seedlings were watered with deionized water so any mercury found in control seedlings roots probably came from inoculants and shoots probably from absorb mercury vapours circulating in the air. Mercury treatments were randomly placed with dissimilar treatments adjacent to each other. Since mercury is very volatile, the vermiculite/sand media may have allowed volatilization of mercury affecting all sycamore seedlings. So control seedlings leaves and soil media were exposed to mercury vapours circulating in the surrounding air.

Due to unequal variance of both root and shoot data, rank transformation was performed (Table 3.2(a) and 3.3 (a)). Inoculation, and the interaction between inoculation and mercury had no significant effect on shoot mercury concentration, but mercury treatment significantly ($P < 0.0001$) affected shoot components. When we examine mercury treatment difference on shoot mercury, there was no treatment difference between CH_3HgCl or $CH_3HgCl + Hg(NO_3)_2$, but $Hg(NO_3)_2$ and control treatments were different from each other, and from treatment containing CH_3HgCl (Table 3.2(b) and 3.3 (b), Fig. 3.2 and 3.3). Mercury treatment ($P < 0.001$) and inoculation treatment ($P = 0.02$) had a significant effect on root mercury concentration. When we examine mercury treatment difference in roots, the control (no mercury) treatment differed from all others. For inoculation treatments 1D + 2A and 2A differed from the other treatments.

There are several factors that affect the amount of metal absorbed by the roots and subsequent translocation to the shoots; (1) Metal concentration and

speciation in the soil solution, (2) metal movement from bulk soil to root surface and into the root, and (3) metal translocation from root to shoot. Several studies have suggested that translocation of mercury from the roots of plants to their shoot is minimal, and mercury accumulation in foliar components occurs from

Table 3.2. Type 3-test of fixed effects for roots, (b) mercury least square means for roots and (c) inoculation least square means for roots.

(a)

Effect	Num DF	Den DF	F-value	Pr > F
inoculation	3	14	4.36	0.023
mercury	3	14	22.84	<0.0001
inoculation*mercury	9	14	2.32	0.0763

(b)

Mercury Treatment	LS
CH_3HgCl	1418.28^{A}
$CH_3HgCl+Hg(NO_3)_2$	1384.33^{A}
$Hg(NO_3)_2$	1205.19^{A}
No Mercury	10.71^{B}

(c)

Inoculation Treatment	LS
1D + 2A	1544.06^{A}
2A	985.54^{AB}
1D	793.73^{B}
No inoculate	695.17^{B}

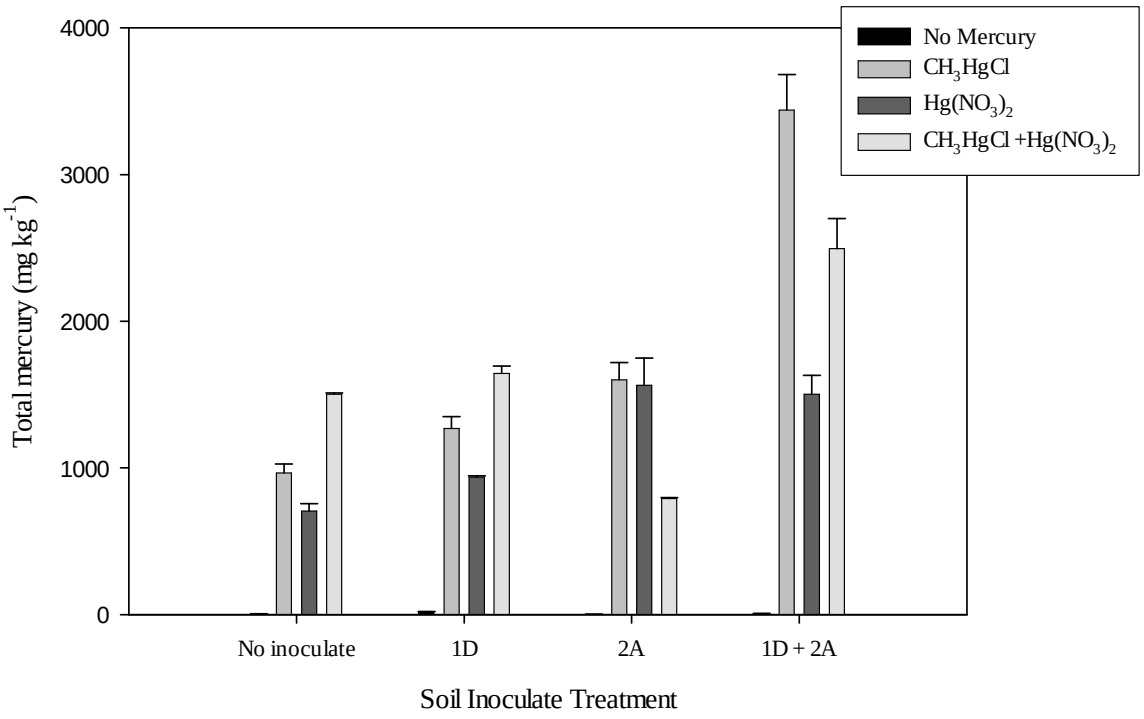


Figure 3.2. Average total mercury concentration (mg Hg kg^{-1}) for sycamore seedlings roots. Control treatment for roots was below 20 mg Hg kg^{-1}. Bars indicate standard error.
Table 3.3. Type 3 -test of fixed effects for shoots, (b) mercury least square means for sycamore shoots.

(a)

Effect	Num DF	Den DF	F-value	Pr > F
inoculation	3	15	1.21	0.3387
mercury	3	15	35.03	<0.0001
inoculation*mercury	9	15	1.3	0.3144

(b)

Mercury Treatment	Least square means
CH_3HgCl	208.02^A
$CH_3HgCl+Hg(NO_3)_2$	191.63^A
$Hg(NO_3)_2$	130.99^B
No Mercury	2.05^C

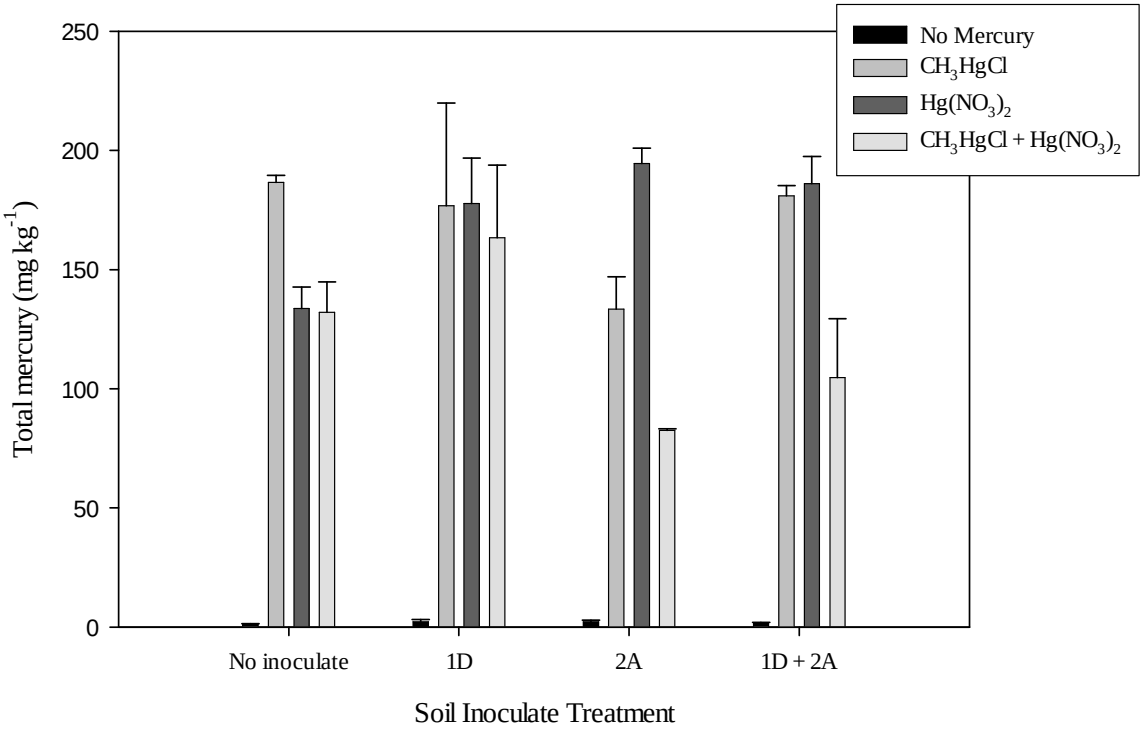


Figure 3.3. Average total mercury concentration (mg Hg kg^{-1}) for sycamore seedlings shoots (stem/leaves). Control treatment for shoots was below 3 mg Hg kg^{-1}. Bars indicate standard error.

soil volatilization and/or atmospheric deposition of mercury (Lodenius, 1994; Gilmour and Miller, 1973; Lindberg et al., 1979; Millhollen et al., 2006). Mercury has been shown to disrupt aquaporins thus blocking the long distance water transport (Hejnowiez and Sieves, 1996). Limitations in water movement may have helped to minimize mercury translocation form root to shoot observed in this study.

Endo- and ecto- mycorrhizal presence and abundance post mercury exposure

Mycorrhizal fungi were present on the roots of sycamore seedlings in all mercuric treatments. Total endomyocrrhizal and ectomycorrhizal fungal counts from each mercury treatment are listed in Table 3.4. There was no significant interaction (P = 0.07) between mercury treatment and mycorrhizal quantity (Table 3.2 (a)). Sycamore seedlings were

initially inoculated with soils obtained from established vegetation plots designated as "high" (> 200 mg kg^{-1}), from 1D and 2A. Prior to inoculation, both endomycorrhizal and ectomycorrhizal fungi were observed in these soil samples. Later, total mercury analyses of soil samples showed actual soil Hg to differ from initial estimates: plot 1D at 216 mg Hg kg^{-1}, and 2A at 82.9 mg Hg kg^{-1} was actually a "high" plot. Background, or natural levels of mercury in soils range from 20 to 625 ng Hg g^{-1} (Agocs, 1992), so soils used to inoculate the sycamore seedlings had at least seven times more mercury than is normally found in the soil environment.

We initial proposed that the inoculation of American seedlings with mercury contaminated soils would enhance their tolerance to mercury. Due to the toxic effects and subsequent termination of all treatments with methyl mercury chloride, a

Table 3.4. Endo- and ecto- mycorrhizal fungal count post greenhouse study.

Inoculation Treatment	Mercury Treatment	Mycorrhizae	
		Endo	Ecto
$Control_1$	$CH_3HgCl+Hg(NO_3)_2$	2	0
$Control_2$	$CH_3HgCl+Hg(NO_3)_2$	0	0
$Control_1$	CH_3HgCl	33	0
$Control_2$	CH_3HgCl	2	0
$Control_1$	No Mercury	1	0
$Control_2$	No Mercury	3	13
$Control_1$	$Hg(NO_3)_2$	1	0
$Control_2$	$Hg(NO_3)_2$	0	0
$1D_1$	$CH_3HgCl+Hg(NO_3)_2$	78	7
$1D_2$	$CH_3HgCl+Hg(NO_3)_2$	100	0
$1D_1$	CH_3HgCl	4	0
$1D_1$	No Mercury	13	0
$1D_2$	No Mercury	0	10
$1D_1$	$Hg(NO_3)_2$	14	0
$1D_2$	$Hg(NO_3)_2$	1	3
$2A_1$	$CH_3HgCl+Hg(NO_3)_2$	6	7

$2A_2$	$CH_3HgCl+Hg(NO_3)_2$	0	34
$2A_1$	CH_3HgCl	13	0
$2A_2$	CH_3HgCl	0	75
$2A_1$	No Mercury	7	0
$2A_2$	No Mercury	0	25
$2A_1$	$Hg(NO_3)_2$	4	0
$2A_2$	$Hg(NO_3)_2$	0	0
$1D_1 + 2A_1$	$CH_3HgCl+Hg(NO_3)_2$	0	0
$1D_1 + 2A_1$	CH_3HgCl	10	10
$1D_2 + 2A_2$	CH_3HgCl	0	6
$1D_1 + 2A_1$	No Mercury	8	0
$1D_2 + 2A_2$	No Mercury	0	25
$1D_1 + 2A_1$	$Hg(NO_3)_2$	6	2

*The subscript indicates which block replicate (1 or 2) in the greenhouse study the mycorrhizal fungal count was from. Two plots 1D, 2A, and a combination of the two, were used to inoculate sycamore seedlings. Harvested roots were plated on slides and counted until 100 counts were obtained.

conclusive statement would be premature. But we did see an increase in mercury accumulation in the roots of sycamore seedlings inoculated with soils from both plot 1D and 2A (Figure 3.2) in both mercury treatments that contained methyl mercury chloride. This was interesting to see, even though a statistical analysis of differences between mercury treatments was not observed. To suggest a synergistic effect of mercury accumulation from fungi in these plots is plausible (Gabriel et al., 1994).

Soil microorganisms play a pivotal role in the mobilization and immobilization of cations and anions, thereby changing their availability to plants (Birch and Bachofen, 1990). Mycorrhizal fungi are soil microorganisms, which establish mutual symbioses with a large majority of higher plants and provide a direct connection between soil and plant roots (Barea and Jeffries, 1995). When a disturbance occurs, mycorrhizal fungi are

important to the survival and establishment of plants and identification of fungal species present in disturbed environments is critical. In this greenhouse study only the abundance and presence of both endomycorrhizal and ectomycorrhizal fungi were assessed but identification of fungal species was not performed. Increased knowledge regarding fungal species presence in heavy metal contaminated soils is important, not only for the shared beneficial mutualism to higher plants but also for the identification of fungal hyperaccumulators in amelioration of contaminated soil.

The effect of mercury treatment on sycamore leaf spectra

There were observable differences in foliage of sycamore seedlings watered with mercury treatments which contained CH_3HgCl versus $Hg(NO_3)_2$ and control treatments. By day 8, early senescence, and leaf wilting was apparent in the leaves of seedlings exposed to CH_3HgCl. After day 11 all CH_3HgCl treatments were terminated due to seedling death. Control and $Hg(NO_3)_2$ treatments remained viable until completion of experiment. Mercuric nitrate treatment had no observable effects on leaf color or health of sycamore seedlings. Deignan and Lewis (1988) investigated the inhibitory effects of nitrate on ammonium absorption in hydroponic culture. Nitrate inhibition of ammonium reduced carbon stress in roots of *Triticum aestivum* allowing for more carbon available for the extension of roots. Nitrogen absorption from soil solution into plant roots can be in the form of nitrate (NO^-_3) or ammonium ions. It is possible that the nitrate dissociating from mercuric nitrate compound allowed for an increased of nitrate uptake through sycamore seedling roots. Analysis of total soil mercury concentration and total nitrogen concentration in plant tissue was not conducted.

The spectra of a healthy plant has characteristic strong absorptions at ~ 450 nm and ~ 680 nm due to chlorophylls and strong reflectance in the NIR arising from internal scattering of light from the cell wall-air interfaces (Wooley, 1971). When vegetation is stressed, spectra show increased reflectance in the visible light range, due to loss of chlorophyll and decreased reflectance in the NIR range, due to damage to leaf cell walls and mesophyll tissue (Boyer et al., 1988; Carter, 1994; Smith et al., 2004). There were differences such as discoloration, wilting and curling of leaves observed especially when sycamore seedlings were watered with any methyl mercury treatment. As mentioned above, the seedlings in treatments that received methyl mercury chloride expired before the end of the experiment, so pigment analyses was conducted only on days 2, 4, 7 and 10. We further examine the effects of each mercury treatment on each wavelength characteristic of photosynthetic pigment by days sampled (Table 3.5). There was an overall significant difference ($P < 0.001$) in all pigment wavelengths sampled (p430, p448, p471, p642, p662, and p680), when we analyzed each treatment by day. Seedlings on day 2, 4, 7 had leaves with pigments (p430, p448, p471, and p680) that were significantly different between $Hg(NO_2)_3$ and CH_3HgCl treatments, and combo and control treatments were significantly different on day 2. Interestingly for chl *a* and chl *b* (p642 and p662), seedlings on day 2 were significantly different ($P< 0.0001$) from day 4, but not from days 7 or 10 for $Hg(NO_2)_3$ and CH_3HgCl treatments. When plants are under stress or during senescence, chlorophyll tends to decline more rapidly than caroteniods (Sims and Garmon, 2002). Pigments are important for leaf function, and variations in pigment content may provide information concerning the physiological state of leaves.

To further examine the effects of mercury on leaf chemistry, we explored spectra in the near infrared region, 1.0 to 2.5 µm (1000-2500 nm) of the mercury treated sycamore leaves by day and mercury treatment. Near-infrared spectra were visually similar, showing two bands at 1443 nm and 1930 nm over the selected spectral range (Figure 3.4). Several possible compounds in plant leaf tissue such as water (with peaks at 1430 and 1930 nm), starch (with peaks at 990, 1220, 1450, 1560, 1700, 1770, 1930 nm) or cellulose (1200, 1480, 1930 nm) could be possible absorptive components present. Water has been shown to mask the absorption features of plant compounds (i.e. cellulose, starch, lignin, etc.) in the NIR (Elvidge, 1990).

Principal component analysis (PCA) was performed only on days 2, 4, and 10 due to a shift in the spectral data on day seven. Analysis was conducted on the whole data set

Table 3.5. Mean reflectance at six wavelengths (p430, p448, p471, p642, p662, p680) for sycamore seedling leaves on days 2, 4, 7, and 10 during mercury treatment.

Day	2	4	7	10
p430				
$Hg(NO_3)_2$	$0.05^{C} \pm 0.01$	$0.15^{A} \pm 0.02$	$0.10^{B} \pm 0.06$	$0.12^{AB} \pm 0.08$
CH_3HgCl	$0.05^{C} \pm 0.01$	$0.14^{A} \pm 0.01$	$0.12^{B} \pm 0.13$	$0.12^{AB} \pm 0.05$
Combo	$0.04^{B} \pm 0.01$	$0.14^{A} \pm 0.01$	$0.18^{A} \pm 0.23$	$0.11^{A} \pm 0.01$
Control	$0.05^{B} \pm 0.02$	$0.16^{A} \pm 0.03$	$0.13^{A} \pm 0.02$	$0.16^{A} \pm 0.13$
p448				
$Hg(NO_3)_2$	$0.05^{C} \pm 0.02$	$0.15^{A} \pm 0.02$	$0.10^{B} \pm 0.06$	$0.13^{AB} \pm 0.08$
CH_3HgCl	$0.05^{C} \pm 0.01$	$0.14^{A} \pm 0.01$	$0.12^{B} \pm 0.14$	$0.13^{AB} \pm 0.06$
Combo	$0.04^{B} \pm 0.01$	$0.15^{A} \pm 0.01$	$0.18^{A} \pm 0.23$	$0.12^{A} \pm 0.01$
Control	$0.05^{B} \pm 0.02$	$0.16^{A} \pm 0.03$	$0.14^{A} \pm 0.02$	$0.17^{A} \pm 0.14$
p471				
$Hg(NO_3)_2$	$0.05^{C} \pm 0.02$	$0.17^{A} \pm 0.02$	$0.11^{B} \pm 0.07$	$0.14^{AB} \pm 0.09$
CH_3HgCl	$0.05^{C} \pm 0.01$	$0.16^{A} \pm 0.01$	$0.14^{B} \pm 0.16$	$0.14^{AB} \pm 0.07$
Combo	$0.04^{B} \pm 0.01$	$0.16^{A} \pm 0.01$	$0.20^{A} \pm 0.23$	$0.13^{A} \pm 0.01$
Control	$0.06^{B} \pm 0.02$	$0.18^{A} \pm 0.04$	$0.16^{A} \pm 0.03$	$0.19^{A} \pm 0.16$

p642				
$Hg(NO_3)_2$	$0.11^{CB} \pm 0.03$	$0.36^{A} \pm 0.06$	$0.22^{B} \pm 0.13$	$0.25^{B} \pm 0.14$
CH_3HgCl	$0.11^{C} \pm 0.02$	$0.36^{A} \pm 0.08$	$0.29^{B} \pm 0.25$	$0.36^{AB} \pm 0.16$
Combo	$0.10^{B} \pm 0.02$	$0.41^{A} \pm 0.07$	$0.43^{A} \pm 0.20$	$0.38^{A} \pm 0.10$
Control	$0.13^{B} \pm 0.03$	$0.40^{A} \pm 0.11$	$0.38^{A} \pm 0.05$	$0.37^{A} \pm 0.21$
p662				
$Hg(NO_3)_2$	$0.07^{CB} \pm 0.02$	$0.23^{A} \pm 0.03$	$0.16^{B} \pm 0.10$	$0.19^{AB} \pm 0.12$
CH_3HgCl	$0.07^{C} \pm 0.02$	$0.23^{A} \pm 0.03$	$0.21^{B} \pm 0.22$	$0.24^{AB} \pm 0.13$
Combo	$0.06^{B} \pm 0.01$	$0.25^{A} \pm 0.03$	$0.30^{A} \pm 0.22$	$0.24^{A} \pm 0.05$
Control	$0.08^{B} \pm 0.02$	$0.26^{A} \pm 0.07$	$0.25^{A} \pm 0.04$	$0.27^{A} \pm 0.19$
p680				
$Hg(NO_3)_2$	$0.07^{C} \pm 0.02$	$0.24^{A} \pm 0.02$	$0.16^{B} \pm 0.10$	$0.19^{AB} \pm 0.11$
CH_3HgCl	$0.06^{C} \pm 0.01$	$0.24^{A} \pm 0.02$	$0.19^{B} \pm 0.21$	$0.21^{AB} \pm 0.11$
Combo	$0.06^{B} \pm 0.01$	$0.24^{A} \pm 0.02$	$0.27^{A} \pm 0.23$	$0.21^{A} \pm 0.02$
Control	$0.07^{B} \pm 0.02$	$0.25^{A} \pm 0.05$	$0.25^{A} \pm 0.04$	$0.27^{A} \pm 0.19$

Means and their corresponding letters are listed. Means with the same letter labels are not significantly different.

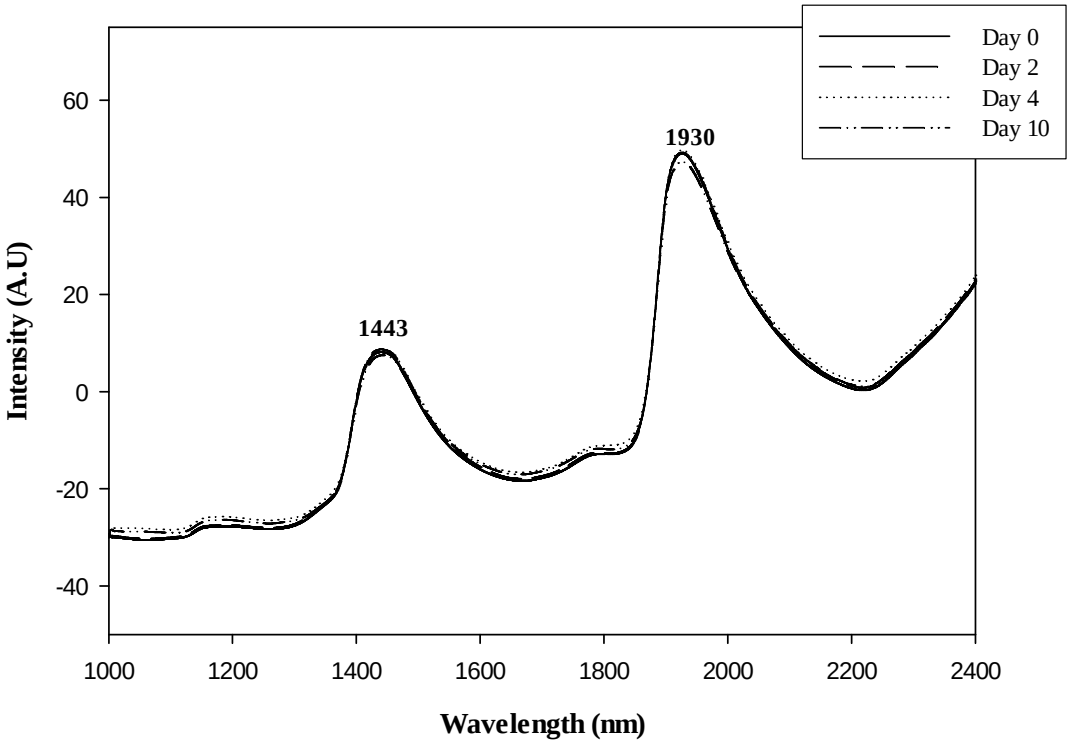


Figure 3.4. Near-infrared spectra averaged across sampling days, from leaves of sycamore seedlings from baseline (day 0, 2), $Hg(NO_3)_2$, CH_3HgCl, and $Hg(NO_3)_2$ + CH_3HgCl (day 4, 10).

to determine variations between mercury-treated and control seedlings (Figure 3.5).

Following mercury treatment, on day 2, there were no differences in mercury-treated and

control leaves indicating that mercury treatments had not affected seedling tissue. But on day 4, there seemed to be a chemical change in all leaves when analyzed by NIR. Even though control seedlings received deionized water, mercury was found in the roots (< 16 mg kg^{-1}) and shoots (leaves and stems) (< 3 mg kg^{-1}) of these seedlings when analyzed post experiment. Control treatments were randomly placed alongside mercury treatments to ensure the same experimental regime. All treatments were administered as a solution where sycamore seedlings were randomly watered.

Mercury is very volatile in air, so when room temperature is greater than 30ºC, the concentration of mercury vapour in the air in greatly increased. The levels of mercury in control seedlings were minute as compared to mercury treated seedlings, where roots had concentrations of > 700 mg kg^{-1} and shoots had concentrations > 75 mg kg^{-1} mercury. Even though the mercury levels in the control seedlings were significantly lower than in mercury-treated seedlings, NIR spectroscopy was not sensitive enough to detect chemical changes following mercury application. Finally, when sycamore leaves were analyzed by the NIR probe, several leaves were burned, which may have caused chemical changes in the plant leaf tissue.

Conclusion

Some plants are known to concentrate Hg as less-toxic chemical forms such as elemental Hg droplets or as HgS (mercury sulfide) (IUPAC, 1998). American sycamore seedlings were able to concentrate a considerable amount, 4.9 to 3266 mg kg^{-1} of

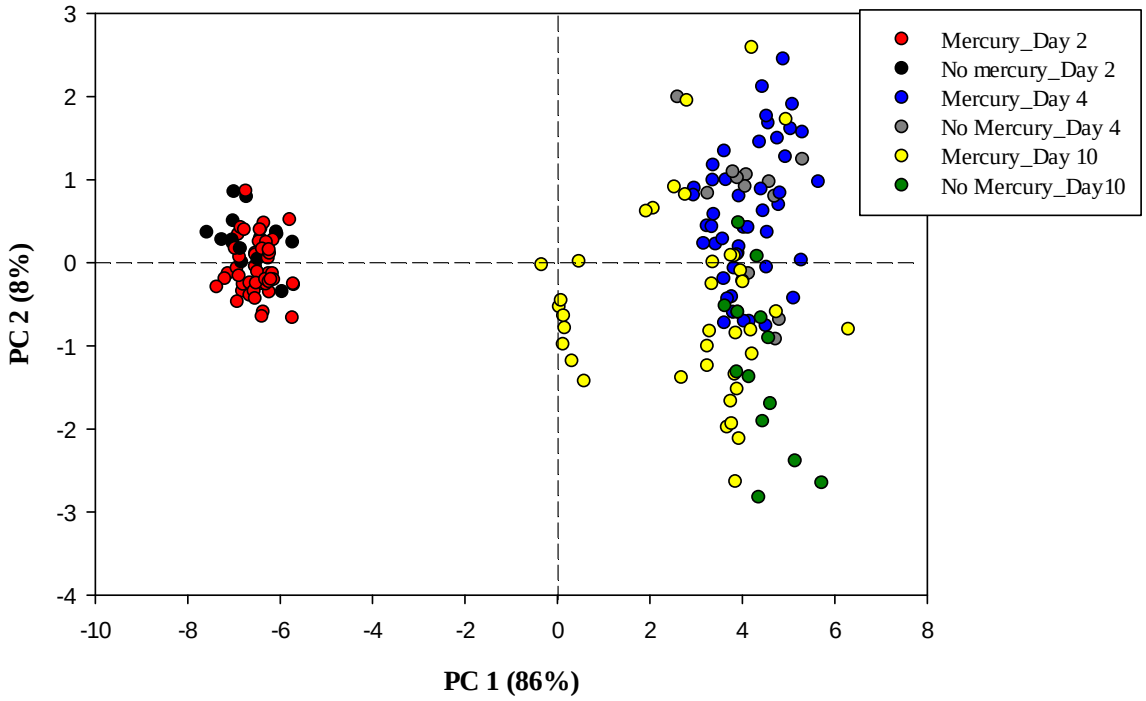


Figure 3.5. Principal component analysis (PCA) score plot of spectra from leaves treated with deionized water, $Hg(NO_3)_2$, CH_3HgCl and $Hg(NO_3)_2$ + CH_3HgCl.

mercury, in their roots even when associated with mycorrhizal fungi. Inoculation enhanced the accumulation of mercury in the roots especially when different soils

contaminated with mercury were combined, suggesting synergism. The stem and leaves of sycamore seedlings had concentrations of mercury 1.0 to 242.2 mg kg^{-1}, which was considerably less than was present in roots. NIR spectroscopy was able to detect chemical changes in mercury treated sycamore seedlings but due to the volatility of mercury, seedlings in control treatments also acquired dry deposition of mercury via soil volatilization.

Reference

Agerer, R. (1991). "Characterization of ectomycorrhiza. In Norris JR, Read DJ, Varma AK (eds). ." Techniques for the study of mycorrhiza: Methods Microbiology **23**: 25-73.

Agocs, M. (1992). Mercury toxicity. Atlanta, GA, Atlanta, GA : U.S. Dept. of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Auge, R. M and Moore, J. L., 2005. Arbuscular mycorrhizal symbiosus and plant drought resistance. *In*: Mehrotra VS, editor. Mycorrhiza: role and applications. New Dehli, Allied Publishers Limited, pp. 136-162.

Barceleo, L., Poschenrieder, C. (1990). "Plant water relations as affected by heavy metal stress." A Review Journal Plant Nutrition **13**: 1-37.

Barea J. M., Jeffries, P. (1995). Arbuscular mycorrhizas in sustainable soil plant systems. In: Hock B, Varma A, editors; Hock B, Varma A, editors. Mycorrhiza structure, function, molecular biology and biotechnology. Heidelberg, Germany: Springer-Verlag; pp. 521–559.

Birch L. D., Bachofen, R. (1990). Effects of microorganisms on the environmental mobility of radionucleides. In: Bollang J M, Stozky G, editors; Bollang J M, Stozky G, editors. Soil Biochemistry. Volume 6. New York, N.Y: Marcel Dekker; pp. 483–527.

Beauford, W., Barber, J., Barrington, A. R. (1977). "Uptake and distribution of mercury within higher plants." Physiologia Plantarum **39**: 261-265.

Bland, J. M., Altman, D. G. (1995). "Multiple significance test: the Bonferroni method." Statistics Notes **310**: 170.

Burger, J., Campbell, K., Campbell, T., Shukla, T., Dixon, C., Gochfeld, M. (2005). "Use of Central Stonerollers (Cyprinidae: *Campostoma anomalum*) From Tennessee as a Bioindicator of Metal Contamination " Environmental Monitoring and Assessment **110**: 171-184.

Campbell, K., Campbell, T., Burger, J. (2005). "Heavy Metal Concentration in Northern Water Snakes (*Nerodia sipedon*) from East Fork Poplar Creek and the Little River, East Tennessee, USA." Archives of Environmental Contamination and Toxicology **49**: 239-248.

Colpaert, J. V., Van Assche, Jozef A. (1992). "The effects of cadmium and the cadmium-zinc interaction on the axenic growth of ectomycorrhizal fungi." Plant and Soil **145**: 237-243.

Deignan, M. T., Lewis, O. A. M. (1988). "The inhibition ammonium uptake by nitrate in wheat." New Phytologist. **110**: 1-3.

Dunagan, S. C., Gilmore, Martha S., Varekamp, Johan C. (2007). "Effects of mercury on visible/near-infrared reflectance spectra of mustard spinach plants (*Brassica rapa* P.)." Environmental Pollution **148**: 301-311.

Elvidge, C. (1990). "Visible and near infrared reflectance characteristics of dry plant material." International Journal of Remote Sensing **11**: 1775-1795.

Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. M., and Siegel, B. Z. (1983). "Removal of Uranium (VI) from Solution by Fungal Biomass and Fungal Wall-Related Biopolymers." Science **219**: 285-286.

Gabriel, J., Mokrejs, M., Bily, J., Rychlovsky, P. (1994). "Accumulation of heavy metals by some wood-rotting fungi." Folia Microbiology **39**: 115-118.

Gilmour, J. T., Miller, M. S. (1973). "Fate of a Mercuric-Mercurous Chloride Fungicide Added to Turfgrass." Journal of Environmental Quality **2**(1): 145-148.

Godbold, D. L., Huttermann, A. (1986). "The Uptake and Toxicity of Mercury and Lead to Spruce (*Picea Abies Karst.*) Seedlings." Water, Air, and Soil Pollution **31**: 509-515.

Godbold, D. L. (1991). "Mercury-Induced Root Damage in Spruce Seedling." Water, Air, and Soil Pollution **56**: 823-831.

Hejnowiez, Z., Sievers, A (1996). Journal of Plant Physiology **147**: 516-520.

Hoalgand and Arnon, (1938). The water culture method for growing plants without soil. California Agriculture Experiment Station Circle. 347.

Lindberg, S. E., Jackson, D.R., Huckabee, S.A. Janzen, J.A., Levin, M.J., Lund, J.R. (1979). "Atmospheric emission and plant uptake of mercury from agricultural soils near the Almaden mercury mine." Journal of Environmental Quality **8**: 572-578.

Lodenius, M. (1994). Mercury in Terrestrial Ecosystems: A Review. Mercury Pollution. Boca Raton, CRC Press.

Pacovsky, R. S. (1986). "Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans." Plant and Soil **95**: 379-388.

Patra, M., Sharma, A (2000). "Mercury toxicity in plants." The Botanical Review **66**(3): 379-421.

Prasad, D. D., Prasar, A. R. (1987). "Effect of lead and mercury on chlorophyll synthesis

in mung been seedlings."Phytochemistry **26**: 881-883.

Rogers, R. D., Williams, S. E. (1986). "Vesicular-Arbuscular Mycorrhiza: Influence on Plant Uptake of Cesium and Cobalt." Soil Biology and Biochemistry **18**: 371-376.

Schlegel, H., Godbold, D. L., Hutterman, A. (1987). "Whole plant aspects of heavy metal induced changes in CO2 uptake and water relations of spruce (*Picea abies*) seedlings." Physiologia Plantarum **69**: 265-270.

Schutzendubel, A., Polle, A. (2002). "Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization." Journal of Experimental Botany **53**: 1351-1365.

Sims, D., Garmon, J. (2002). "Relationship between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages" Remote Sensing of Environment **81**: 337-354.

Wang, B., Qui, Y.-L. (2006). "Phylogenetic distribution and evolution of mycorrhizas in land plants" Mycorrhiza **16**: 299-363.

PART IV
DETECTION OF MERCURY AND OTHER METALS IN MERCURY CONTAMINATED SOILS USING MID-INFRARED SPECTROSCOPY

Sharon R. Jean-Philippe, Nicole Labbé, Jennifer A. Franklin*, and Amy Johnson

University of Tennessee, Department of Forestry, Wildlife and Fisheries, 274 Ellington Plant Science Knoxville, Tennessee 37996-4563

*Address correspondence to author. Phone: 865-9742724; email: jafranklin@utk.edu

Abstract

Conventional methods for detection of total soil Hg in contaminated environments are based on time-consuming sample preparation and costly sample analysis. The possibility for prediction of total soil –Hg concentration and other elements in contaminated soils using the mid-infrared (MIR) region (4000 – 600 cm^{-1}) has been investigated. Principal component analysis (PCA) was used to identify patterns or differences in soil spectral data and partial least squares (PLS) was used to develop concentrations of several metals in soil samples. Detection of chemical differences in mercury-contaminated soil samples, and calibration models for Hg and several other elements was achieved using PCA and PLS-2. Pearson correlation identified nine elements (Sr, Ni, Cu, Cd, V, Ti, Fe, Ba, Rb) and total carbon that were significantly correlated with total soil-Hg. Our calibration models showed high *r* for Hg, and Sr ($r \geq .90$) and relatively moderate *r* for Cu and Ni ($r > .80$). Low variation in metal concentrations can cause prediction errors, so mercury concentration in soil samples < 18 mg kg^{-1} was over-predicted in the calibration and validation models. Results support the conclusion that the mid-infrared region could aid conventional method analyses of soils heavily contaminated with certain heavy metals.

Introduction

Mercury pollution has become an important current issue due to its environmental effects at a global scale. Solubility, mobility, and bioavailability of mercury in terrestrial and aquatic ecosystems are strongly controlled by mercury speciation in both soil solution and solid-phase components (1). Mercury transport and distribution in the environment originate from natural (i.e. outgassing, wild fires, geothermal surfaces or volcanoes) and anthropogenic sources (i.e. fossil fuels, smelting lead, zinc ores) (2-5). Various species of mercury exist in soils (i.e. elemental mercury, organic and inorganic mercury) and their potential toxicity depends on their concentration and species present in the soil solution (5). Mercury in soils is firmly bound to organic matter or precipitated as sulphide, and found in trace concentration in soil solution (6).

Due to anthropogenic and natural input, many watersheds have been affected by mercury discharged into waterways such as creeks, lakes and streams. One such case is East Fork Poplar Creek (EFPC) in Oak Ridge, TN, where in the 1950's and 1960's large amounts (~ 1,080 metric tons) of mercury compounds were discharged into EFPC from the U S. Department of Energy's Oak Ridge Tennessee Site (1,5,7). Levels of total mercury in the floodplain soils along EFPC in 1984 ranged from 0.5 to 3000 mg kg^{-1} (5), in contrast to uncontaminated soils across the United States in which total mercury concentration ranged from 0 to 0.2 mg kg^{-1} (8). In addition to mercury discharged into unlined landfills, ponds and streams, other significant contaminates such as strontium, tritium, uranium, technetium and plutonium have been released within this watershed.

Conventional methods for total mercury detection in soil samples require harsh chemicals such as tin chloride ($SnCl_2$), sodium borohydride ($NaBH_4$), or aqua regia (Cl-H.H-N-O_3) in acid solutions which do procreate additional waste, which also can be labor intensive process, and quite costly. These methods include inductively coupled plasma-mass spectrometry (ICP-MS), cold vapor atomic absorption spectroscopy (CVAAS) and atomic emission spectroscopy (AES). The detection limit for mercury for each method mentioned above is 0.001 ppb, and is limited to quantifying total elemental concentrations (9, 10). Infrared spectroscopy can be used to identify many types of organic and inorganic compounds in the form of solids, liquids and gases. The infrared region is divided into three regions: the near-, mid- and far-infrared. In particular, the mid-infrared (MIR) region, with wavenumber from 4000 cm^{-1} to 600 cm^{-1}, can be used to obtain reflectance spectra from a variety of opaque samples (fabrics, soil, powders, polymers, etc.,) with minimum sample preparation by using an Attenuated Total Reflectance (ATR) accessory.

Soils are heterogeneous, with most soil properties dependent on mineral and soil organic matter composition (11). Various soil components such as smectite, kaolin, illite, clays, quartz, and organics (protenaceous, aliphatic, lipid, carboxyl and aromatic compounds) absorb in the mid-infrared and near-infrared (NIR) region, and exhibit spectral patterns with peaks at vibrational frequencies of molecular functional groups (11). Quartz and kaolinite clays occur in more compact soil with high bulk density, and have strong MIR spectra around 1100 – 1000 cm^{-1} (Si-O) and 3690 – 3620 cm^{-1}, respectively. Soil organic matter has spectral peaks due to alkyl –CH_2 at 2930-2850 cm^{-1},

spectral peaks for protein amide OC-NH around 1680 and 1530 cm^{-1}, carboxylate anion COO^- at 1600 and 1400 cm^{-1}, and carboxylic acid COOH around 1720 cm^{-1} (11). The prediction of mercury and several major and trace elements by near-infrared and mid-infrared spectroscopy has previously been reported (11), the calibration for Hg was poor, R^2 0.109 and root mean square error of 0.033, with no independent validation test set. It should also be noted that Hg concentration in soil samples were very low (0.01 to 0.7 mg kg^{-1}). The authors concluded that near-infrared and mid-infrared were not adequate techniques for reducing the need for conventional analysis of soil samples at this concentration range.

The objective of this study was to examine the potential use of MIR in the quantitative determination of total mercury in soil samples within the floodplain of a mercury contaminated site. The concentrations of multiple elements in these soil samples were determined by inductively coupled plasma (ICP), Hg was determined by cold vapor atomic absorption spectroscopy, and the resulting data were used to build and validate models using MIR spectra of the samples.

Experimental Section

Soil Sampling**.** During June 2007, three blocks B1 (N 36° 00.52′, W 084° 14′), B2 (N 36° 00.33′, W 084° 16.82′) and B3 (N 35° 95.243′ W 084° 38.19′) were established along East Fork Poplar Creek (EFPC) and perpendicular to the creek bank. The Natural Resources Conservation Service classified soils along EFPC as Inceptisols. These Newark silt loams are somewhat poorly drained and are moderately acid to moderately alkaline. Historical data for soil total Hg concentration along EFPC obtained from

Science Application International Corporation (12) was used to stratify sampling within each block. Three Hg contamination levels, low (0-50 mg kg^{-1}), medium (50-200 mg kg^{-1}) and high (>200 mg kg^{-1}) were established within each of the blocks, and plots were randomly placed within each of these areas. Plot size was 168.1 m^2 (0.017 hectare), with blocks 1 and 3 having 3 plots each for low and medium levels, and one high level. Block 2 was smaller in area, and had one of each contamination level represented. Due to remediation efforts along EFPC during the late 1980's, there were few areas to establish high-Hg plots. Three 30.48 cm depth soil cores were randomly collected from each plot and bulked, then frozen for later analysis.

Soil Sample Preparation, ICP and Mercury Analysis. All soil samples were air dried for 2 days and finely ground and sieved through a 250 µm (60 –mesh) sieve. Soils were air dried to prevent volatization of mercury than can occur above 30 ˚C. Total elemental analysis was determined by inductively coupled atomic emission spectrometry (ICP) following Nadkarni (13), using microwave oven digestion with modifications. Exchangeable Ca, K, Mg, Pb, Cd, Mn, Fe, Zn, Ni and Cu were determined by ICP after extraction with 1 N ammonium acetate (pH 7.0) and concentrated ammonium hydroxide (14). Cation exchange capacity was determined by extraction with 2 N ammonium followed by distillation (15). Total nitrogen (N) and carbon (C) was determined on a Thermo Eager 3000 analyzer. Soil pH was measured on a1:1 volume soil:water solution.

Samples were analyzed for total mercury concentration by cold vapor atomic absorption spectroscopy (CVAAS) by Western Kentucky University, Institute of

Combustion Science and Environmental Technology. Only soil samples collected in October of 2008 were analyzed for total mercury.

Spectral Analysis. Ground and sieved (~60 mesh) soil samples were pelleted using the Carver Hydraulic Press (Carver, Inc.). Briefly, 0.3 g of each soil sample were placed in a KBr die, and pressed for 4 minutes at 2.26 tonne. Three pellets were made for each soil sample with a pellet thickness of 1.0 mm. Pellets were scanned in the mid-IR on a Spectrum One (PerkinElmer Instruments LLC, Shelton, CT) FT-IR Spectrometer equipped with a Universal ATR. Pellets were scanned from 4000 to 600 cm^{-1} at 1 cm^{-1} resolution with 64 scans per spectrum. A total of 12 spectra were collected per pellet.

Statistical Analysis and Chemometrics. Pearson product moment correlation coefficient (16) was used to identify correlations between CEC, pH, average C, N, Hg, Al, Ba, Cd, Cr, Cu, Fe, K, Mn, Nd, Ni, Sr, Ti, V, Zn, Pb, Rb, S and cations (Ca, Mg, Na, P). Multivariate analyses of the mid-infrared soil spectral data were performed using principal component analysis (PCA) and partial least-squares (PLS) in Unscrambler v. 9.2 software (CAMO Software Inc., Woodbridge, NJ). PCA identified patterns in spectral data allowing the clustering of data and reducing the number of dimensions without the loss of information. All spectra were modified by reduction (averaging) of wavenumber by 4, averaging of the initial sample set by 3 (n = 360 to n = 120), and mean normalized. Additional spectral pretreatments using first and second derivatives with a 9 point smoothing point average were tested. PCA was run initially on the full spectral range (4000-600 cm^{-1}) to characterize soil spectra and detect outliers before establishing the regression model. But due to a lack of apparent spectral features above 1400 cm^{-1}, data

above 1600 cm $^{-1}$ was removed for all further analyses. PLS is a bilinear modeling method where spectral data (X-variables) were projected onto a small number of PLS components. In estimating the latent variables, the response variables (Y-variable(s)) were used to ensure that the first components were the most relevant for predicting the Y-variables (17). There are two versions of PLS regression algorithm, where the PLS1 examines one y-variable and PLS2 examines multiple y-variables. Multiple y-variables (elements) were significantly correlated to total soil mercury, so a PLS2 was performed to developed calibration and validation models for each correlated element. Even though the number of samples to produce an appropriate multivariate model for k (> 3) variables (k is the number of variables) should contain a minimum of $6(k + 1)$ spectra, here we perform a cross validation. Cross validation can be a more efficient way of utilizing small sample numbers. There are two types of cross validations, segmented cross validation and full cross validations. Even though segmented is a faster method, here we choose full cross validation which improves the relevance and power of the analysis. To ensure that the calibration and validation models produced were appropriate for each y-variable analyzed the regression coefficients for each x-loading were compared for each. The parameters used to the qualify the results were the correlation (r), root mean square error of calibration (RMSEC) and root mean square error of validation (RMSEV).

Results and Discussion

Soil Analytes**.** Table 4.1 shows the summary statistics for the analytes examined for soil sampled from EFPC. Total mercury ranged from 0.70 to 758.65 mg Hg kg^{-1}. In addition to mercury, strontium (Sr), tritium (T), uranium (U), technetium (Tc) and plutonium (Pu)

were discharged into EFPC from the Y-12 Facilities. Nine metals (Ba, Cd, Cu, Fe, Ni, Rb, Sr, Ti, V), and carbon were positively correlated to $p < 0.05$, total soil mercury. The majority of metal ions significantly correlated to mercury are transition metals (Cd, Cu, Fe, Ni, Ti and V) and being "class B" metals (Pearson classification) tend to associate with each other. Furthermore the absorption of metal ions at the solid/aqueous solution is controlled exclusively by the "free" metal ion concentration, but also by much stronger adsorbed hydroxo, sulfato, carbonato and other metal complex species (18). Soil samples obtained from EFPC are Newark series consisting of somewhat poorly drained soils formed in mixed alluvium and moderately acid to alkaline. Soil pH was significantly positively correlated ($p < 0.01$) to total soil mercury and ranged from 6.22 to 8.04. Metal ions hydrolyzed onto various oxides as well as other surfaces tend to follow a common trend with solution pH (19). So minimum to no adsorption is observed with low pH (19), where as depending on the metal under observation abrupt increases in adsorption over an narrow pH range due occur (20). Five of the metals (Ba, Cd, Cu, Ni, and Ti) positively correlated with mercury were also positively correlated to pH.

Mercury (Hg^{2+}) ion exhibits a high affinity for sulfide especially in anoxic waters and sediments (21). Schuster (6) found that mercury in soils is firmly bound to organic matter or precipitated as sulfide, and found in trace concentrations in soil solution. Sulfur content in plots along EFPC sampled ranged from 0.16 to 0.59 mg kg^{-1} and was not significantly correlated to total mercury. Barnett and Turner (22) sampled 20 soils from EFPC and found a significantly correlation ($p < 0.0001$) between sulfur and total mercury

Table 4.1. Summary of analytes from EFPC soils**.**

Analyte	Minimum	Maximum	Mean
Al_{ppm}	57.05	127.15	94.84
Ba_{ppm}	61.15	114	83.62
$C_{total\ mg\ g^{-1}}$	0.63	2.34	1.51
Ca_{ppm}	59.3	355.2	169.92
Cd_{ppm}	0.02	0.05	0.027
Cr_{ppm}	0.04	0.19	0.097
Cu_{ppm}	0	0.25	0.096
Fe_{ppm}	13.88	58.65	40.76
Hg_{ppm}	0.7	758.65	103.04
K_{ppm}	14.43	31.19	19.43
Mg_{ppm}	7.74	25.45	16.19
Mn_{ppm}	0.28	3.87	1.51
$N_{total\ mg\ g^{-1}}$	0.04	0.13	0.092
Na_{ppm}	4360	9300	5315.4
Nd_{ppm}	0.02	0.05	0.034
Ni_{ppm}	0	0.19	0.058
P_{ppm}	0.48	2.05	1.21
Pb_{ppm}	0.05	0.14	0.11
Rb_{ppm}	0.04	0.16	0.113
S_{ppm}	0.16	0.59	0.39
Sr_{ppm}	0.04	0.08	0.048
Ti_{ppm}	2.47	4.23	3.31
V_{ppm}	0.04	0.12	0.082
Zn_{ppm}	0.02	0.4	0.177
CEC	0.13	12.09	7.77
pH	5.66	8.04	7.12

*CEC (meq/ 100g soil) and pH were analyzed for 16 soil samples (except 2A).

content but not with total carbon. Total sulfur and mercury concentration in plots sampled ranged between 50 – 1700 mg kg^{-1}, and 15 to 2630 mg kg^{-1}, respectively. The difference in sulfur and mercury totals was related to sampling depth, with deeper soil samples having higher sulfur and mercury concentrations. The authors did not examine other potential analytes. Besides mercury other several other natural and artificial heavy metal contaminates were also released in EFPC such as strontium (Sr), plutonium (Pu), tritium ($_1H_2$), uranium (U), ruthenium (Ru), and technetium (Tc). Certain metals have been shown to influence one another's uptake in animals and plants and toxicity in natural ecosystems (23). Mercury has been shown to influence lead and zinc (24) and iron (25) uptake and/or toxicity in invertebrates in aquatic environments. In terrestrial environments organic matter and iron oxides are two important mercury sorbents (26). Higher organic C also resulted in absorption of Hg in soil (27). Kinniburgh and Jackson (28) reported that roughly more than 90% Hg (II) was absorbed by iron (Fe). Iron and carbon are two of several elements in this study shown to be significantly correlated to mercury.

MIR Analysis. The mid-infrared spectra were collected from 10 (1B, 1D, 1E, 1G, 1H, 3A, 3C, 3D, 3F, 3G) of the 17 plots sampled. Problems occurred with seven soil samples during pressing so these were not analyzed. The same absorbance spectra patterns were observed for all soil samples. The prominent absorbance bands across all samples were 695, 779, 795, 911, 1030, and 1167 cm^{-1}. The strong band at 675-995 cm^{-1} was due to C-H stretching vibration. The C-H showed bands at 675, 779, 795, and 911 cm^{-1}. The strong band 1050-1300 cm^{-1}

was due to C-O stretching vibration. The C-O showed bands at 1030 and 1167 cm^{-1}. The soil spectra of the highest (1D, 216.2 mg kg^{-1}Hg) and lowest (3C, 0.705 mg kg^{-1} Hg) total mercury are shown in Figure 4.1. The first two principal components of the PCA account for the greatest amount of total variation (98%). Plots where total soil mercury concentration < 7 mg kg^{-1} Hg clustered together and include, 1B (0.945 mg kg^{-1} Hg), 3C (0.705 mg kg^{-1} Hg), and 3F (6.33 mg kg^{-1} Hg) (Figure 4.2 (a)). Principal component 1 (PC1) contains 94% and principal component 2 (PC2) contains 4% of the observed variation. The loadings of the first two principal components are shown in figure 4.2 (b). The important bands responsible for classification of soil samples are 995, 911, 791, 775, 691 cm^{-1} are assigned to C-H stretching vibrations, and 1167, 1123, 1063, 971, 927, 847, 775, and 695 cm^{-1} are assigned to C-H and C-O stretching vibrations.

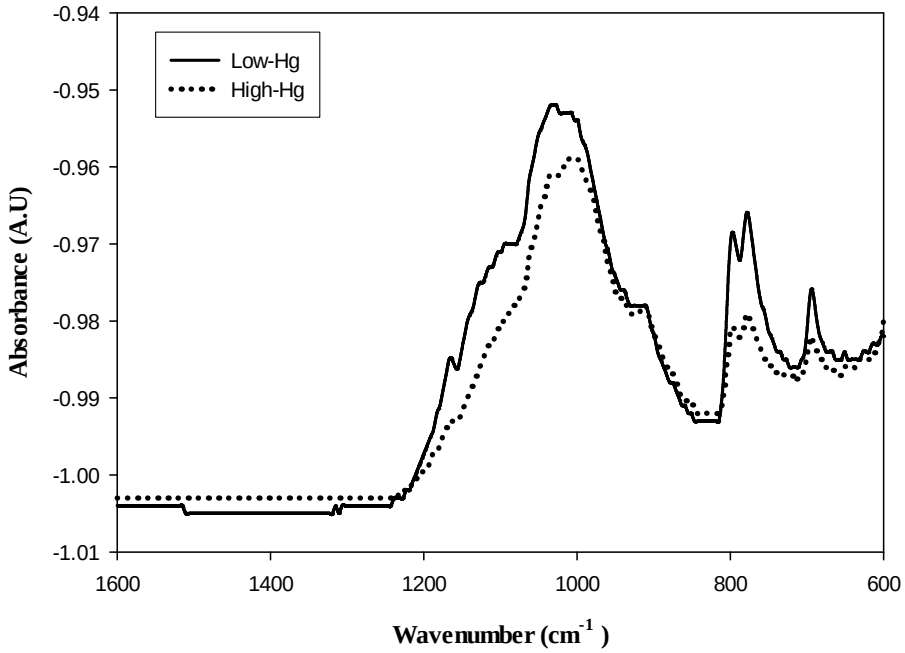


Figure 4.1.Mid-infrared spectra of the highest and lowest mercury soil plots along EFPC.

The Pearson correlation shows nine (Ba, Cd, Cu, Fe, Ni, Rb, Sr, Ti, V) of the twenty-two metals and total carbon were significantly correlated to soil total-Hg concentrations. Due to the significant correlation of these metals and carbon with mercury, we investigated the ability to determine these analytes in soils using a multivariate regression technique, PLS-2, which analyzes multiple y-variables with spectral information. Table 4.2 summarizes the results for the calibration models developed with mid-infrared spectra. The best models (high *r*, low RMSEC and RMSEP) to predict Hg and Sr ($r \geq .90$), Cu and Ni ($r > .80$), Fe, Cd, C_{total}, Rb, V, Ti, and Ba ($r < .80$) are obtained with general pretreatment methods using no additional pre-processing methods such as 1^{st} or 2^{nd} derivatives (data not shown).

Figures 4.3, Hg (a), Sr (b), Cu (c), and Ni (d) show the relevant regression coefficients for the highest *r* calibration and validation models obtained for these four metals. Examination of results for Hg, where sample distribution was marginal, showed some low (< 21 mg kg^{-1}) samples to be poorly determined with several samples at values ~ 6%, to have ~21% Hg content in the calibration (Fig.4.3a). The validation (*r*) set resulted in over-prediction of several samples, ~6% to be over-predicted by ~ 20% Hg content. Results for Cu were similar, several samples < 1% were slightly over-predicted to have ~2% Cu content in both calibration and validation sets (Fig. 4.3c). Even though Hg and Cu calibration and validation models were $r > 90$ and 80, the ability for the models to capture relatively low concentrations in these samples was poor.

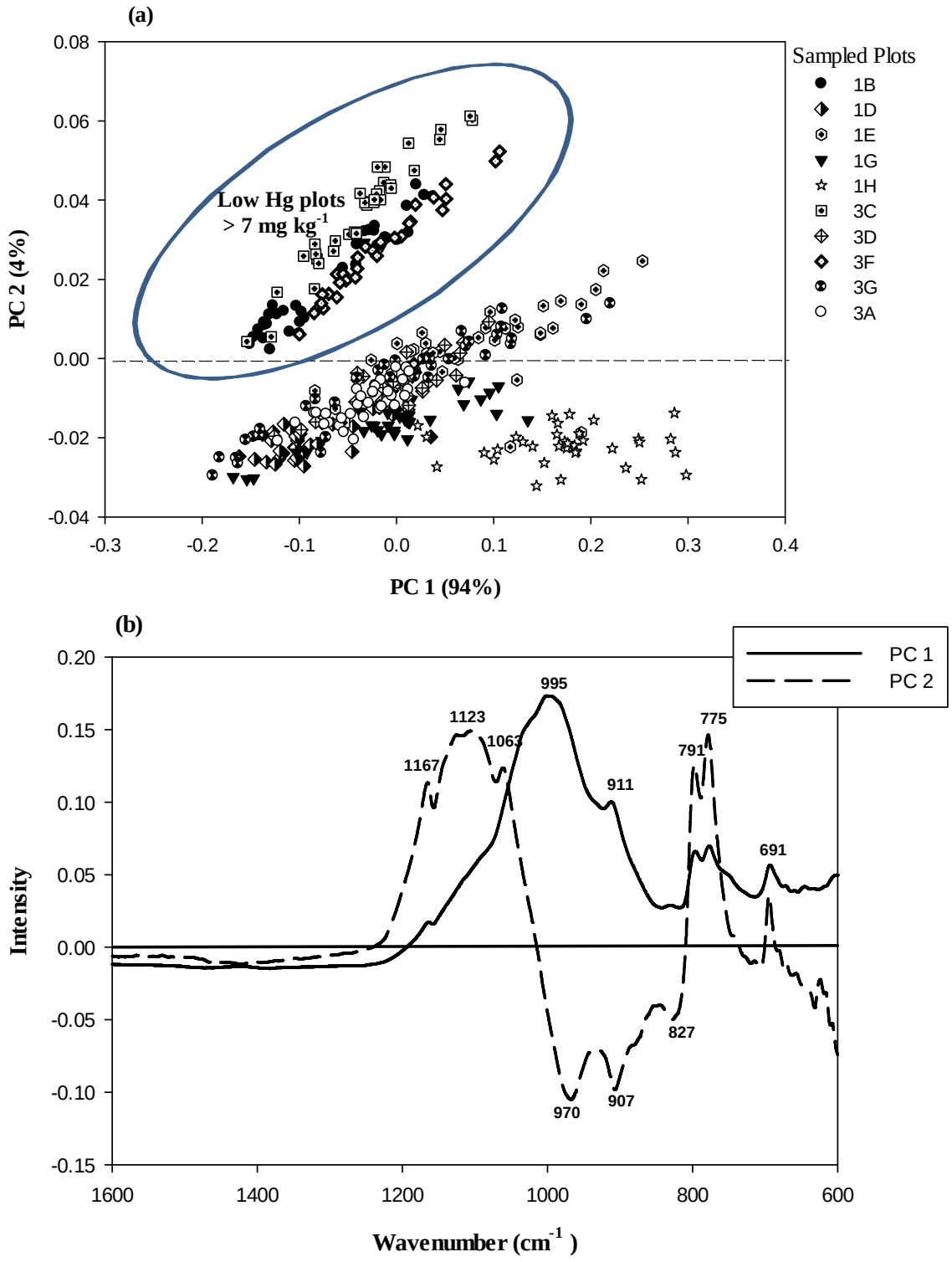


Figure 4.2. (a) PCA score plot of MIR spectra. (b) PC 1 and PC 2 loading plots for 10 mercury soil plots sampled.

Table 4.2. PLS-2 models developed for Hg, Sr, Ni, Cu, Cd, Fe, V, Ti, Rb, Ba, Ctotal

				Full cross-validation			
Element			***Pre-treated**	**Calibration**		**Validation**	
	Min	Max	no. PC	r	RMSEC	r	RMSEP
Hg_{ppm}	0.7	758.65	5	0.93	22.7	0.91	25.8
Sr_{ppm}	0.04	0.08	3	0.90	0.003	0.88	0.003
Cu_{ppm}	0.001	0.25	3	0.87	0.02	0.85	0.03
Ni_{ppm}	0.004	0.19	5	0.86	0.01	0.82	0.01
Fe_{ppm}	13.88	58.65	5	0.79	7.49	0.76	7.9
$C_{total\ mg\ g^{-1}}$	0.63	2.34	3	0.78	0.29	0.76	0.3
Cd_{ppm}	0.02	0.05	4	0.78	0.003	0.76	0.004
Rb_{ppm}	0.04	0.16	3	0.75	0.02	0.74	0.02
Ti_{ppm}	2.47	4.23	5	0.7	0.23	0.69	0.25
V_{ppm}	0.04	0.12	5	0.64	0.01	0.6	0.01
Ba_{ppm}	61.15	114	5	0.52	8.90	0.39	9.7

*All spectra were averaged and mean normalized.

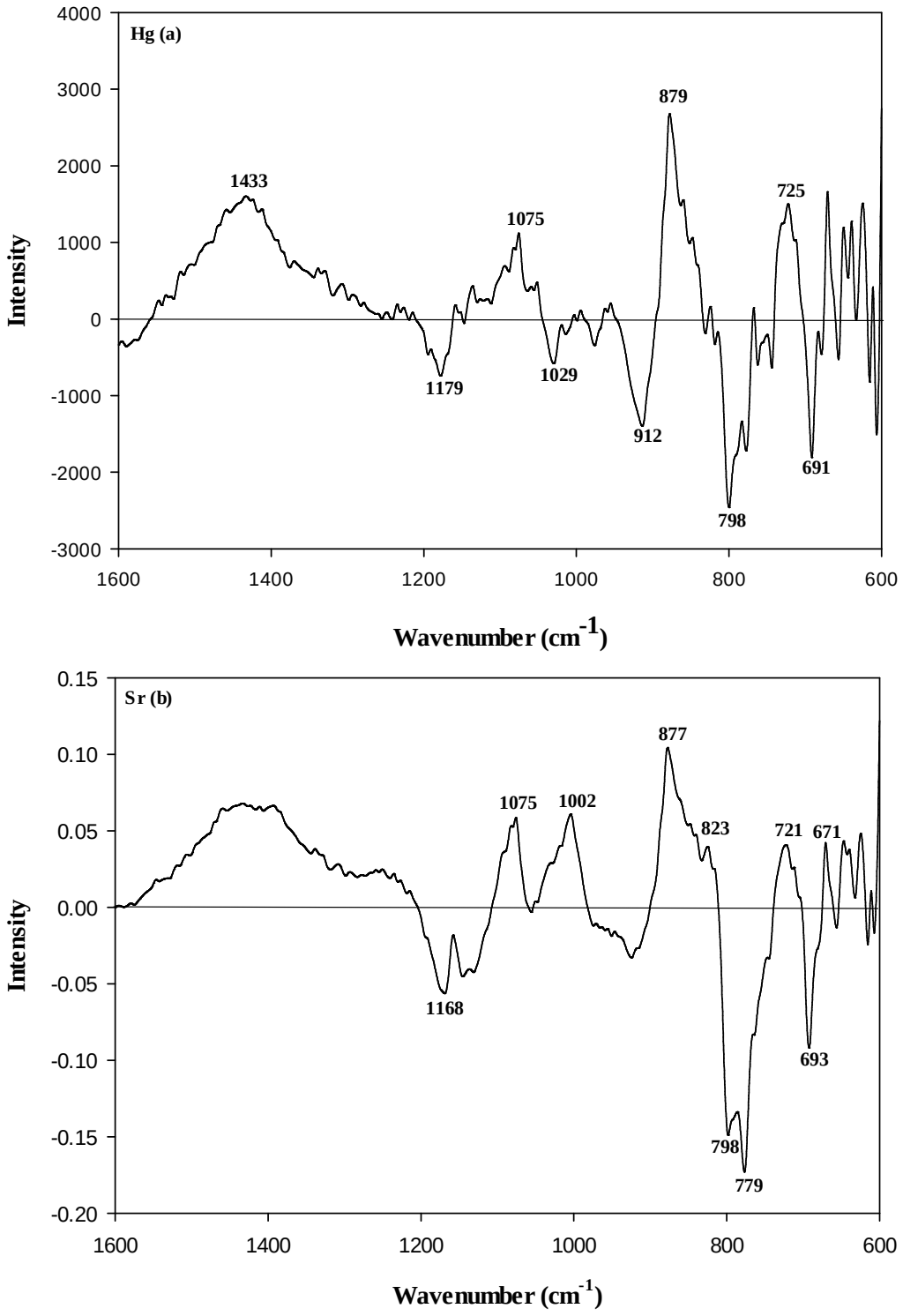
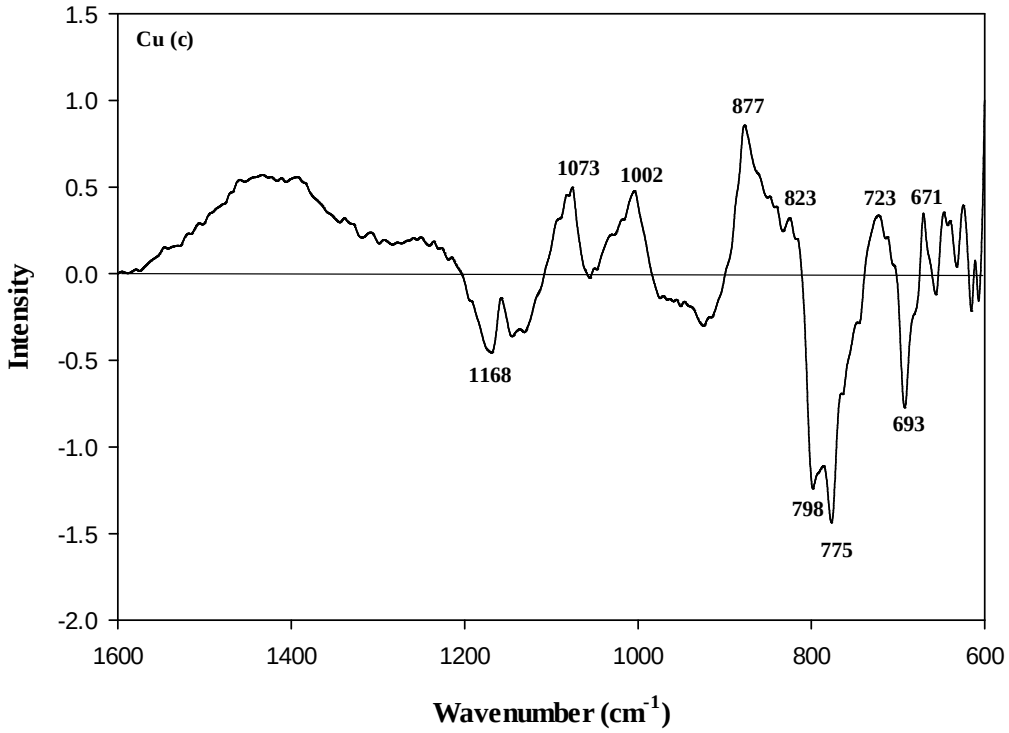


Figure 4.3. Regression coefficients for Hg (a), Sr (b), Cu (c) and Ni (d).

Figure 4.3. Continued regression coefficients.



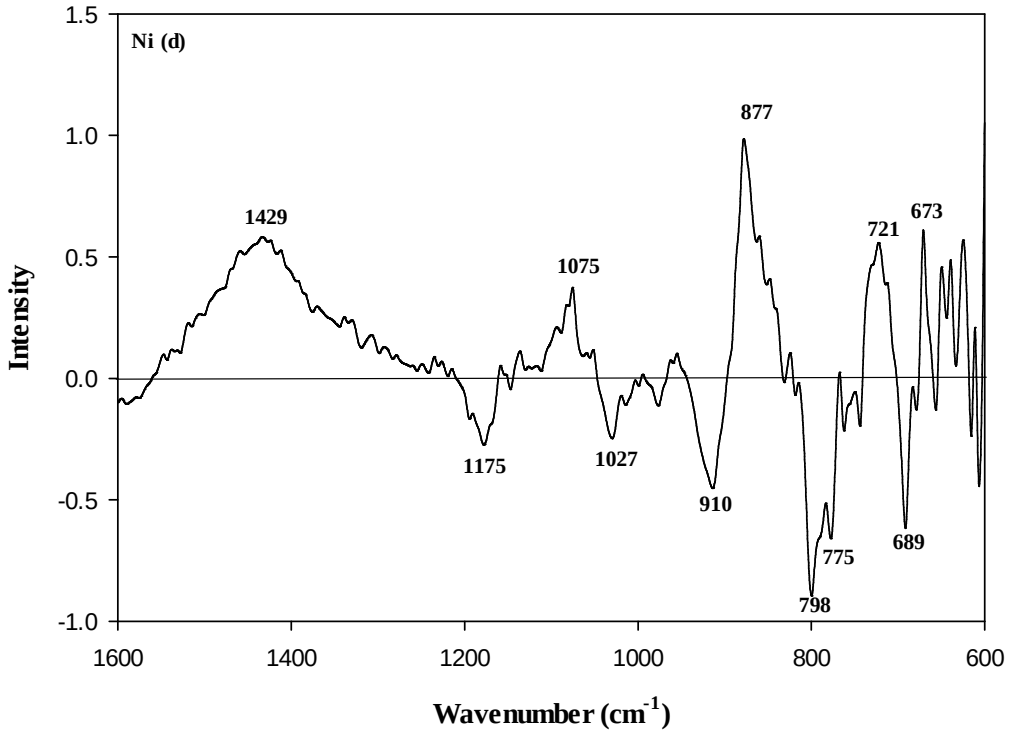


Table 4.3. Regression coefficients for Fe, C, Cd, Rb, Ti, V, and Ba

Element	Regression coefficients (cm^{-1})
Fe	1168 1075 1002 877 823 796 77 5 723 689 671
C	1168 1075 1002 925 877 823 796 775 721 689 668
Cd	1177 1075 1029 977 912 877 823 800 775 723 691 671
Rb	1168 1129 1054 1022 877 825 800 775 721 691 673
Ti	1179 1150 1073 1031 954 912 875 823 800 775 721 689
Ba	1433 1133 1029 1010 979 912 877 802 721 687
V	1433 1137 1073 1077 1006 977 912 877 831 818 802 766 743 723 689 675 668 654

The calibration results for Sr and Ni were fairly accurate in predicting these metals (Table 4.2). The majority of the metals analyzed did not consist of a wide range of concentration, which made it difficult to predict. Regression coefficients for metals with models < 80 are listed in Table 4.3. Reeves and Smith (29) reported that due to the diversity of samples (> 700) there was an inability to capture a relevant calibration and validation models for most metals sampled. The authors did report good calibration models for Ni or Mg when sample concentrations were < 100 mg kg^{-1}. The calibration and validation *r* for Ti and Ba were below < 80 but these models still were able to detect these metals (Table 4.2). Several researchers have developed calibration models for Fe, Cu, Ni, Cd (29, 30) and total carbon (31), using the mid- and near-infrared region. Strong stretching and vibrational bands at 1340-1470 cm^{-1}, 675-995 cm^{-1} and 1030-1167 cm^{-1} indicates C-H and C-O bonds, respectively. The majority of analytes sampled had prominent bands in these regions.

Inaccurate prediction could be made if metal concentrations are below certain levels as seen in Hg and Cu calibration and validation models, due to over-fitting of spectral data. Reeves and Smith (29) noted that a larger sample size may reduce the problems associated with over-fitting. The *fingerprint* region 1200 to 600 cm^{-1} is very useful in identifying small differences in the structure and constituents of molecules of interest (32). A close match between several spectra in this fingerprint region constitutes strong evidence for the identity of compounds in the spectra. Several analytes (Hg, Sr, Ni, Cu, Cd, Ti, and C_{total}) had sharp bands between this region (691, 726, 783, 798, 799, 726 cm^{-1}). The majority of single bonds give rise to absorption bands within these frequencies and due to similarities in their energies, strong interactions occur between neighboring bonds. Absorption is thus a composite of competing interactions and depends on the overall structure of the molecule (32). Exact interpretation of spectra in this region poses a problem due to the complexity of the spectra, but it is the complexity that leads to unique structure and eventual identification.

Acknowledgments

Financial support for this research was provided by Southern Regional Education Broad (SREB). Thanks to the Department of Energy (DOE) Oak Ridge, TN for access to sampling sites. Also special thanks to Nicolas Andre, Cassi Jones and Ryan Blair for assistance with sampling preparation and technical support.

Reference

(1) Han, F. X.; Su,Y. M.; David, L.; Waggoner, C. A.; Plodinec, M. J. Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. *The Science of the Total Environment* **2006**, *368* (753).

(2) Boening, D. W. Ecological effects, transports, and fate of mercury: a general review. *Chemosphere* **2000,** *40* (1335).

(3) Schroeder, W. H.; Munthe, J. Atmospheric Mercury- An Overview. *Atmospheric Environment* **1998,** *32* (809).

(4) Nriagu, J. O.; Pacyna, J. M. Quantitative assessment of worldwide contamination of air, water and soils by trace metals." *Nature* **1988,** *333* (134).

(5) Revis, N. W.; Osborne, T. R.; Holdsworth, G.; Hadden C. Distribution of Mercury Species in Soil from a Mercury-Contaminated Site. *Water, Air, and Soil Pollution* **1989,** *45* (105).

(6) Schuster, E. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes- a review of the literature. *Water, Air, and Soil Pollution* **1991,** *56* (667).

(7) Bashor, B.; Turri, P. A method for determining an allowable concentration of mercury in soil. *Archives of Environmental Contamination Toxicology* **1986,** 15 (435).

(8) Trost, P.B.; et al. Distribution of mercury in residual soil. In *Environmental Mercury Contamination*; Hartung. R., Dinman, B.D., Eds.; Ann Arbor Science Publisher: Michigan 1970; pp 178-196.

(9) Clevenger, W. L.; Smith, B. W.; Winefordner, J. D. Trace Determination of Mercury: A Review. *Critical Reviews in Analytical Chemistry* **1997,** *1* (1).

(10) Han, F. X.; Patterson, W. D.; Xia, Y.; Sridhar, B.B. Rapid Determination of Mercury in Plant and Soil Samples using Inductively Coupled Plasma Atomic Emission Spectroscopy, A Comparative Study. *Water, Air and Soil Pollution* **2006,** *170* (161).

(11) Janik, L. J.; Merry, R. H.; Skjemstad, J. O. Can mid infrared diffuse reflectance analysis replace soil extraction? *Australian Journal Experimental Agriculture* **1998,** *38* (681).

(12) SAIC. East Fork Poplar Creek-Sewer Line Beltway Remedial Investigation Report: Addendum. Oak Ridge, TN, *U.S. Department of Energy* **1994**.

(13) Nadkarni, R. A. Applications of microwave oven sample dissolution in analysis. *Analytical Chemistry* **1984**, *56* (2233).

(14) Sumner, M. E.; et al. Cation exchange capacity and exchange coefficients. In *Methods of Soil Analysis. Part 3. Chemical Methods*; Soil Society of America and American Society of Agronomy Book Series 5; Madison, WI; 1996; pp 1201.

(15) Annual Book of ASTM Standards, Part 31, *Water*, Standard DI426-74, Method A, 1976; p 237.

(16) SPSS, 2008. SPSS for Windows, rel. 11.0.1 2001. Chicago: SPSS Inc.
(17) Martin, M.; Labbè, N.; Rials, T.; Wullschleger, D. Analysis of preservative-treated wood by multivariate analysis of laser-induced breakdown spectroscopy spectra. *Spectrochimica Acta Part B*: *Atomic Spectroscopy* **2005,** *60* (1179).
(18) Elliott, H. A.; Huang, C. P. The effect of complex formation on the adsorption characteristics of heavy metals. *Environmental International* **1979,** *2* (145).
(19) Guy, R. D.; Chakrabarti, C. L.; Schramm, L. L. The application of a simple chemical model of natural waters to metal fixation in particulate matter. *Canadian Journal of Chemistry* **1975** *53* (661).
(20) James, R. O.; Stiglich, P. J.; Healy, T. W. Analysis of models of adsorption of metal at oxide/water interfaces. *Transactions of the Faraday Society* **1973,** *59* (142).
(21) Morel, F.M.; Kraepiel, A.M.L.; Amyot, M. The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecological Systematics* **1998,** *29* (543).
(22) Barnett, M. O.; Turner, R. R. Bioaccessibility of Mercury in Soils. *Soil and Sediment Contamination* **2001**, *10* (301).
(23) Casini, D.; Depledge, M. H. Influence of copper, zinc, and iron on cadmium accumulation in the Talitrid amphiphod, *Platorchestia platensis*. *Bulletin Environmental Contamination Toxicology* **1997,** *59* (500).
(24) Gray, J. S. *Pollution and Physiology of Marine Organisms.* Academic Press, London, 1974.
(25) Fowler, B. A.; Wolfe, D. A.; Hettler W. F. Mercury and iron uptake by cytochromes in mantle epithelial cells of quahog clams (*Mercenaria mercenaria*) exposed to mercury. *Journal of Fish Resources Broad of Canada* **1975,** *32* (1767).
(26) Gabriel, M.; Williamson, D. Principal geochemical factors affecting the speciation and transport of mercury through the terrestrial environment. *Environmental Geochemistry and Health* **2004,** *26* (421).
(27) Miretzky, P.; Jardim, W.F.; Bisnoti, C.M.; Rocha, C. J. Factors affecting Hg(II) adsorption in soils from the Rio Negro basin (Amazon). *Quim Nova* **2005,** *28* (438).
(28) Kinniburgh, G. G.; Jackson, M. L. Absorption of Mercury (II) by Iron Hydrous Oxide Gel. *Soil Science Society of American Journal* **1978,** *42* (45).
(29) Reeves, J.; Smith, D. The potential of mid- and near- infrared diffuse reflectance spectroscopy for determining major- and trace elements concentrations in soils from a geochemical survey of North America. *Applied Geochemistry* **2009,** *24* (1472).
(30) Malley, D. F.; Williams, P. C. Use of near-infrared reflectance spectroscopy in prediction of heavy metal in freshwater sediment by their association with organic. *Environmental Science & Technology* **1987,** *31* (3461).
(31) Michel, K.; Ludwig, B. Prediction of model pools for a long term experiment using near-infrared spectroscopy. *Journal of Plant Nutrient and Soil Science* **2010,** *173* (55).
(32) Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*; Harcourt Brace & Company, Orlando, FL, 1998.

PART V CONCLUSIONS AND IMPLICATIONS

Overview

The Oak Ridge Reservation located in Oak Ridge, Tennessee, was established back in the 1940's by The Atomic Energy Commission. Their main focus was enrichment of uranium and the construction of the atomic bomb. Through the years of operation, significant quantities of uranium, technetium, plutonium, mercury, and fission products were dumped into unlined landfills, settling ponds and surface streams. Several water ways were impacted by the pollution, one case is East Fork Poplar Creek located in Anderson and Roane Counties, Tennessee. EFPC headwaters begin at the Oak Ridge Y-12 facility, and travels offsite through the city of Oak Ridge, emptying into the Clinch River and Watts Bar Lake. Estimations of total mercury released into the environment through the soil and EFPC are over 700,000 pounds. The forest plant, fungal and soil communities established along EFPC were initially exposed to an acute toxic dose of mercury and other metals for roughly ten to fifteen years (1950-1965). This acute toxic exposure probably was lethal to established seed banks, certain tree species, grasses, terrestrial and aquatic animals, and microbial communities and caused leaching of important elements (nutrients) from the soil solution.

The goal of this research project was to evaluate and document the effects of mercury and other heavy metals on forest community composition. I first conducted a field study to investigate tree species diversity; identify and quantify mycorrhizal presence; and determine the physiochemical composition of soils post-heavy metal exposure. Secondly, I evaluated the ability of several tree species (*Platanus occidentalis*, *Acer rubrum*, *Pinus echinata* and *Pinus taeda*) to germinate in mercury solution and determined the lethal and non-lethal concentration limits for each species tested. Thirdly,

I determined whether the mutualistic association shared between *Platanus occidentalis* seedlings and mycorrhizal fungi enhance tolerance to mercury exposure by conducting a greenhouse study. The total mercury concentration in roots and shoots of seedlings was quantified, and analysis of chemical changes in leaves due to mercury exposure by near-infrared (NIR) spectroscopy was investigated. I finally used mid-infrared (MIR) spectroscopy to analyze mercury contaminated soils, by developing calibration and prediction models from soil spectra data collected by MIR and analyzed these data using multivariate techniques. Implications and future efforts in the study of heavy metal pollution of forest community structure is discussed below.

Implications

Both natural and anthropogenic impacts resulting in heavy metal contamination may affect the vitality and stability of forest ecosystems. Often these impacts influence species diversity of terrestrial and aquatic plants and animals, reduce soil microbial communities, and alter nutrient loads. The presence of pollutants in the environment will either affect the area occupied by the species or resources used by species present, depending on the tolerance or avoidance mechanisms of the species (Salminenan et al., 2001). Unfortunately, the delicate balance between members in a community will be disturbed as pollutants eliminate the abundance of more sensitive populations (Hernandez and Pastor, 2008).

Soils are the main terrestrial source for metal pollutants (Hernandez and Pastor, 2008) with several metals affecting each other's toxicity and uptake in animals and plants in natural ecosystems (Casini and Depledge, 1997). The identification of species adapted to soil environments polluted with heavy metals can be important in remediation and

reclamation efforts on polluted sites. The investigation reports the presence of several tree species established within the contaminated floodplains of East Fork Poplar Creek. I was not able to detect relationships between soil heavy metal concentration and tree species diversity and richness. Several plausible adaptations to heavy metal contaminated environments have been suggested by other authors which include: intrinsic properties in plants and/or mutualistic association shared by plants and mycorrhizal fungi, excretion of H^+ to change soil pH, excretion of exudates such as organic acids or phytosiderophers in the rhizosphere, or increased production of reactive oxygen species due to oxidative stress (Polle and Rennenberg, 1993). Probably through these and other avoidance or tolerance mechanisms, plant and fungal species have been able to thrive on my study site. But to better characterize tree species composition, fungal abundance and presence and heavy metal concentration, sample size would greatly need to be increased, especially for such a diverse environment.

I further investigated heavy metal exposure on seed germination to see whether established seed banks can be affected by acute or chronic heavy metal exposure. The ability to germinate in any mercury solution was species, compound, and concentration dependent. However, there were differences between species in germination rate and early seedling survival under high mercury levels, suggesting that genetic (hormones) and phenotypic (seed coat, size, weight) differences can play a definite role species response to heavy metal exposure. This strongly suggests that moderate remediation of soils is sufficient, due to the fact that germinating seeds, plants, and fungal species are present in environments that still contain relatively high levels of pollution as in the case of EFPC.

To better understand the ability of plant/fungal species to thrive in heavy metal contaminated environments in a more controlled setting, I conducted a greenhouse study to investigate the effects of different soil inoculants on the tolerance of sycamore seedlings to heavy metal (mercury) exposure. Inoculated seedling roots did accumulate greater amounts of mercury compounds in their tissue than non-inoculated plants. Also, the combination of different soil inoculates resulted in greater accumulation of mercury in seedling roots. This suggests the synergistic effect of fungi in the accumulation of mercury. Mycorrhizal fungi associated with plants do accumulate heavy metals, which may result in positive or negative effects to the host plant. The accumulation of metals has been reported in the fruiting bodies and mycelium of ectomycorrhizae (Leyval, et al., 1997), and in the extraradical hyphae of endomycorrhizal species (Cooper and Tinker, 1978). The ability for mycorrhizal plants to sequester and translocate metals into their tissue is very important because increasing exposure of heavy metals in the environment poses a threat to the food chain, with risk to human health (Schutzendubel and Polle, 2002). If mycorrhizal associations results in a greater amount of mercury and other heavy metals in above-ground tissue, movement into upper levels of the food chain will be enhanced.

Traditionally, conventional methods for detecting heavy metals in contaminated samples are labor intensive and relatively expensive. My results show that quantitative analysis based on NIR and MIR spectroscopy could be used to develop calibration models that can use spectral data to distinguish contaminated from uncontaminated samples. The analysis of plant leaves by NIR resulted in detection of foliar water changes in mercury-exposed plants, but the use of this technique may be limited due to damage to

plant leaves by the NIR probe, which may cause chemical changes in the plant leaf tissue. The development of calibration and validation models from soil spectral data was hindered by tremendous heterogeneity of soils, due to soil type, horizons sampled, and land use.

Future efforts need to be made in the development of calibrations based on plant leaf response to mercury and other heavy metals, to further investigate the changes in leaf spectra from metal application. Such models could be used to monitor the elemental content of reclaimed soils prior to planting regimes, and thereafter to ensure the safety of food to animals and humans.

References

Casini, D., Depledge, M. H. (1997). "Influence of copper, zinc, and iron on cadmium accumulation in the Talitrid amphiphod, *Platorchestia platensis*." Bull Environmental Contamination Toxicology **59**: 500-506.

Cooper, K. M., Tinker, P. B. (1978). "Translocation and transfer of nutrients in vesicular-arbuscular mycorrhiza. II Uptake and translocation of phosphorus, zinc, and sulphur." New Phytologist **81**: 43-52.

Hernandez, A. J., Pastor, J. (2008). "Relationship between plant biodiversity and heavy metal bioavailability in grasslands overlying an abandoned mine." Environmental Geochemistry Health **30**: 127-133.

Leyval, C., Turnau, K., and Haselwandter, K. (1997). "Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects." Mycorrhiza **7**: 139-153.

Polle, A., Rennenberg, H. (1993). Significance of antioxidant in plant adaptation to environmental stress. In: Mansfield, T., Fowed, L., Stoddard, F., eds. Plant adaptation to environmental stress. London: Chapman and Hill. pp. 263-273.

Salminenan, J., Gestel, C. A., Oksanen, J. (2001). "Pollution-induced community tolerance and functional redundancy in a decomposer food web in metal-stressed soil." Environmental Toxiclogy and Chemistry **20**: 2287-2295.

Schutzendubel, A., Polle, A. (2002). "Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization." Journal of Experimental Botany **53**: 1351-1365.

VITA

Sharon Rose Sparks Jean-Philippe was born the 8th of thirteen children in Atlanta, Georgia, in 1980. Sharon graduate from Benjamin E. Mays High School in 1998. She then went to study at Tennessee State University where she graduated with a Bachelor's in Biology and a minor in Chemistry in 2002. She accepted a Graduate Assistant position in the Botany department at The University of Tennessee, Knoxville in 2002. Studying under the tutelage of Dr. Karen Hughes she completed her Master's in Botany where she investigated the "Antioxidant Properties of Some Edible Fungi in the Genus *Pleurotus*."

In the fall of 2006, Sharon began her doctoral work with Dr. Jennifer Franklin. Here primary research interest in understanding how plant and fungal communities adapt to soil environments polluted with heavy metals has lead her to pursue a professional career in natural resources.