REMOVING ARSENIC AND LEAD FROM SOILS USING A BIOREMEDIATION APPROACH: DESIGN, IMPLEMENTATION AND ANALYSIS OF A FEASIBILITY STUDY ON VASHON ISLAND, WASHINGTON

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ABSTRACT

This thesis explores the use of bioremediation techniques at a site contaminated with the heavy metals arsenic (As) and lead (Pb) on Vashon Island, WA in the South Puget Sound. Soils around the South Puget Sound are contaminated with toxic quantities of As and Pb left by an American Smelting and Refining Company (ASARCO) smelting plant that operated for 100 years in Ruston/Tacoma, WA. This thesis reviews the history of soil contamination by ASARCO and the relevant literature on bioremediation methods. A feasibility study was then conducted to remove As and Pb using bioremediation methods. This study used organic *Brassica juncea* plants (mustards) that accumulate soil As and Pb into their tissues. To promote plant growth and metal accumulation, a commercially available blend of microorganisms called SCD BioKlean™, and biochar (organic charcoal) were added. The performance of this 3-component guild was compared to different combinations of these same components (mustards alone, BioKlean™ + mustard, and biochar + mustards) to determine the optimal treatment. Experiments were conducted in 16, 1m square plots with 4 plots devoted to each of the 4 treatments, plus 2 control plots. The hypothesis tested was that the most effective treatment would be the 3-component guild. The As and Pb concentration in the soils were analyzed for total metals by Freidman and Bruya Inc., a professional laboratory, before and after the treatments were applied. To obtain funding two proposals were written and both received funding. The feasibility study was conducted over one growing season from the end of May through September 2011 on Vashon Island. There was a high rate of mustard mortality 3 weeks after sprouting in all of the plots without biochar. The biochar may have contributed to the survival of mustards by retaining moisture and by providing nutrients for growth not found in the un-amended soil. The soil metal concentrations showed great variability before (As = 81.33 ± 37.7 s.d. Pb=145.56 ± 111.20 s.d.) and after treatments (As = 71.13 ± 27.33 s.d. Pb= 104.06 ± 55.98 s.d.) indicating large heterogeneity in the soil composition. While decreases in metal concentration over time occurred in most plots, statistical tests (t-test and Wilcoxon sign rank sum) showed that only the 3-element guild and biochar + mustard treatments significantly reduced Pb. There were unexpected increases in metal concentration in some plots. This suggests transportation processes that moved the soils/metals during the study into and out of the plots. Most plots showed a significant difference between before and after applications, even in the control plots. This could be a result of tilling that was applied to all plots. On the basis of this preliminary data, suggestions for future research are provided plus improved experiments with fewer variables and increased sample size are presented. This study and the general bioremediation approach are discussed in the broader context of finding practical, cost-effective solutions to detoxify soil in South Puget Sound. Appendices include grants written to fund this study.

Key words: phytoremediation, bioremediation, arsenic, lead, *Brassica juncea*, biochar, microorganisms

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CHAPTER I Introduction

Figure 1.Tacoma Smelter Photo Courtesy of the Department of Ecology Website

Summary of Thesis Work

The work in this thesis contributes to the efforts of the non-profit organization, Social Ecology Education & Demonstration School (SEEDS), to aid in the removal of heavy metals from soils on Vashon Island. The goal is to develop an inexpensive and replicable way to reclaim contaminated sites and to make areas safe for living and for growing food. This project is part of a larger ongoing soil remediation project called the Home Remedy Project focusing on soil remediation in people's backyards and creating community awareness of contamination issues. This thesis reports the current state of ongoing research with the Bioremediation Home Remedy Project for SEEDS by providing the initial data and results of the first 4 months of research. The thesis consists of a literature review, design and implementation of a feasibility study, and the results and discussion of that study. The later part of this document called *Grounding the Research*, is devoted to other ways I have been involved with the research process such as funding the research, providing ongoing research needs and ideas, and other projects involved with creating awareness around this particular heavy metal contamination issue.

Significance and Broader Impacts

A consequence of industrialization is the increased presence of heavy metals in soils. These have significant and negative consequences to human health, especially for developing children. In the United States the Environmental Protection Agency (EPA) was formed to create stricter standards and regulations on air, water, and soil pollution. The goal of the EPA when it formed in 1970 was to repair the damage already done to the environment and work to prevent further damage to the environment. This agency and others have been working together to combat lingering pollution, but there is still a great deal of work to be done. Heavy metal pollution occurring around older smelters can be experienced far after the operation has been terminated because these metals are in their most elemental form preventing any further breakdown into a non-toxic state. "Heavy metals cannot be destroyed biologically (no "degradation", change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001)."

The lingering heavy metals are one of the worst environmental problems of today. "In the USA alone, more than 50,000 metal-contaminated sites await remediation, many of them Superfund sites (Ensley, 2000 as found in Bennett et al., p. 432 2003)." In 2000, "[s]ixty-four percent of Superfund and Resource Conservation and Recovery Act sites [were] contaminated with both organic and heavy metal species and another 15% [were] contaminated solely by metals (Henry, p.3 2000)." The magnitude of the pollution problem near these sites illustrates the broad application area where bioremediation could be applied.

The Commencement Bay/Nearshore Tideflats Superfund Site was established in Tacoma and Ruston, Washington representing a highly industrialized area with 6 different hazardous waste cleanup sites within its borders including the Asarco Smelter-Ruston Site (EPA Region 10). Despite the work performed to date to clean up this site, current cleanup methods available for communities with contamination are inadequate. Many soils in the surrounding area have not been cleaned up or remediated. When soils are "cleaned up" they are simply transported to landfills and replaced with foreign soils.

The work from this research will further current knowledge of ecologically and socially responsible processes for dealing with heavy metal contamination. A focus of recent research, bioremediation is aimed at using biological processes to remediate pollutions in soil and water. It can be more cost effective than current cleanup methods and actually aims to remediate the soils rather than transport them to landfills where they continue to remain toxic. Bioremediation uses living organisms to naturally re-assimilate, accumulate, or neutralize toxins of all kinds and is argued to be an effective way to actually restore healthy soil properties. If this bioremediation feasibility study provides successful results, it can be applied in many of these contaminated areas.

SEEDS

History and Mission of the Social Ecology Education & Demonstration School (SEEDS)

Headquartered on Vashon Island in Washington State, the Social Ecology Education and Demonstration School (SEEDS) helps meet the urgent need for an educational ecological project aimed at both local and global communities. The mission of SEEDS is to develop and offer educational experiences that enhance people's abilities to knowledgeably and creatively address the interwoven social and ecological stresses of our time. Through an intensive and interdisciplinary study, participants are assisted in gaining a rounded and critical understanding of current approaches to social and ecological reconstruction. Participants are provided opportunities to test various reconstructive strategies by means of individually designed practicum learning experiences.

Beginning in 2009, SEEDS has played a key role in the development of what has come to be known as the Vision for Vashon. A comprehensive community development effort, Vision for Vashon sparked citizen work groups in affordable housing, community health, community solar energy, alternative currency systems, food security, and grassroots sustainability projects. The food security/food sovereignty group has become a particularly active and robust group. SEEDS' goals in relation to food sovereignty include the promotion of collaborative farming/gardening, and education in permaculture approaches. This includes education and research on the heavy metal contamination.

This thesis was done for SEEDS who inspired, aided, and funded the research-based educational project to discern and implement a system for removing these contaminants using bioremediation methods. With SEEDS, our stated priority is that the system be affordable, effective and replicable to other communities. "We will freely share our findings with other areas facing the challenge of contaminated soils, and we anticipate that our research will benefit countless communities in their quest of restoring land for food production and other community needs. (Harris and Frances Block Grant)"

Successful Grant Writing and Reporting

In the Appendix you will find the grant proposal and reports written for Harris and Frances Block Foundation who provided funding for this research and community work with SEEDS. There is also a grant written to the Evergreen Foundation Activities Fund who awarded $2,000 towards sample testing to be conducted at Evergreen by students who were knowledgeable in using an ICP-MS (Inductively Coupled Plasma Mass Spectrometer but wanted to gain more experience on the device for a real world problem.

Background

History of ASARCO

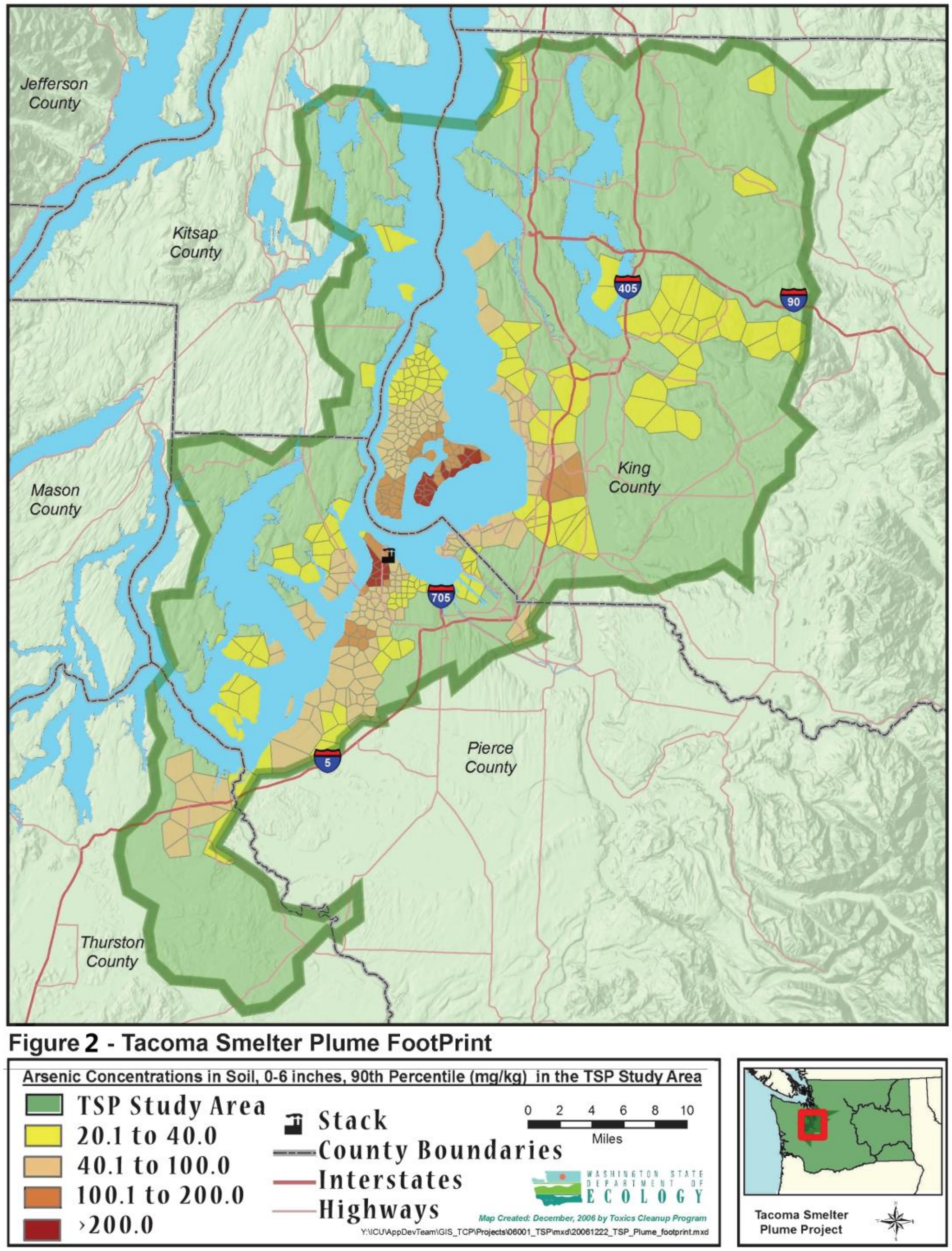
The American Smelting and Refining Company (ASARCO) founded in 1899, is a mining, smelting and refining company that is responsible for 20 superfund sites in the United States. The Commencement Bay/Nearshore Tideflats Superfund Site includes the ASARCO Smelter site in Ruston, Washington (EPA). This smelter, as seen in Figure 1, started operating in 1888 as the Ryan Smelter lead (Pb) refinery (historylink.org). In 1890, it was bought and renamed the Tacoma Smelting and refining Company under William Rust who sold the plant in 1905 for $5.5 million to the American Smelter and Refining Company converting it to a copper smelter in 1912 (historylink.org). ASARCO was founded by Henry H Rogers, William Rockefeller, Adolph Lewisohn, Anton Eilers and Leonard Lewisohn, all big players in the United States western industrialization. ASARCO operated the plant until 1985 when it was closed.

Smelting is the process of separating a particular metal, copper in this case, from impurities contained in mined ore concentrates. Heating the ore concentrate to a high temperature causes the metals to melt. Then the melted metal is smelted to produce a specific metal, copper in this case, or a high-grade metallic mixture along with a solid waste product called slag (Smelting, 2010). "The principal sources of pollution caused by smelting are contaminant-laden air emissions and process wastes such as wastewater and slag (Weiss, 1985)". These contaminants remain long after smelting has been completed.

“Tacoma was the home of the only copper smelter in the nation to use ore with high arsenic content, and accounted for 25 percent of inorganic arsenic emissions nationwide (Tacoma Environment 2010).” Over the 100-year period of operation, some of the byproducts such as Pb and arsenic (As) were released into the air through the smokestack of the ASARCO smelter landing downwind of the site. Annually, around 10,000 tons of As was produced at the ASARCO smelter (Sloan, p14, 2011). These emissions were distributed over 10,000 square miles through a very tall smokestack. The smelter held the record for the tallest building on the west coast for many years:

> The smelter was known for its tall 562 foot smokestack, which sent pollutants up and away from the smelter into surrounding communities. While the smelter was permanently closed in 1986 and the stack demolished in 1993, the environmental damage was already complete. We now know that lead and arsenic pollution was carried by the wind over a wide expanse of King, Pierce, Thurston, and Kitsap counties. (Kingcounty.gov)

Twenty-five years after the smelter was closed, the landscape is still quilted with contamination from the past windfall patterns. (Map below). This map shows As contamination areas. The green outline represents the Tacoma Smelter Plume (TSP) Study area, yellow areas have 20.1-40.0 ppm As, tan has 40.1-100.0 ppm As, orange has 100.0-200.0 ppm As, and red has 200.0 ppm and up. The As concentrations measured in the soils for this map were taken from the top 0-6 inches of the surface soils.



Map Created: December 2006 by Toxic Cleanup Program and shows the different As concentrations (ppm) in the footprint area (Pb has similar fallout)

The EPA designated the ASARCO site as well as the 23 acre slag peninsula created by the plant as part of the Commencement Bay Superfund site in 1979 and stopped copper smelting in 1985 (Commencement Bay/Nearshore Tideflats Superfund Site ROD p2-1). Due to the costs associated with the pollution cleanup, ASARCO filed for protection under Chapter 11 of the United States Bankruptcy Code on August 9, 2005 (Case Number 05-21207, Southern District of Texas, Corpus Christi Division).

The ASARCO bankruptcy was the largest environmental bankruptcy in U.S. history (EPA Compliance). "The EPA, along with other federal and state agencies pursued and received $1.79 billion to fund environmental cleanup and restoration under a bankruptcy reorganization of…ASARCO (EPA Compliance)." The money paid went to fund future cleanup work with the EPA and to pay for natural resource restoration through the Department of Interior and the Department of Agriculture.

History of ASARCO Pollution and Cleanup

The funded cleanup work has enabled the EPA and the Washington State Department of Ecology (WSDOE) to document the contamination of Pb and As in the adjacent areas. WSDOE found concentrations of 360 parts per million (ppm) of As and 1300 ppm of Pb on certain soils on the south side of Vashon Island. This level of contamination is well over the EPA's safe limit for bare soils where actions, such as excavation, must be performed to remediate the problem. Contamination is more than three times the EPA limit for areas with children. It is far above Washington State's limit of 20 ppm concentration standard for As and the state's 250 ppm for Pb (WSDOE).

Table 1. Concentrations of heavy metals allowed by Federal and State Standards.			
EPA As	EPA Pb	Washington State As	Washington State Pb
Excavate if above 230ppm	Excavate if above 530ppm	20ppm	250ppm

The natural background levels of As in soils in Washington are around 7-9 ppm (San Juan, p14). The allowable limit for As contamination is 20 ppm in residential areas (San Juan, 1994). Background levels of Pb in Washington range from 11-24 ppm (San Juan, p14, 1994) while allowable limits are 250ppm.

The Washington State Department of Ecology performed an initial soil test study that looked at the depth profile and reach of the contamination in King, Kitsap, Pierce and Thurston Counties. Glass et al. conducted and reported the first round of sampling concluding soil As and Pb contamination from deposition of airborne particulates creates depth profile in soils is where the contamination remains near the surface and concentrations typically decrease rapidly with increasing depth, especially below 12 inches deep (p52). When contaminated soils are disrupted the contamination patterns become more complex.

> …depth profiles at developed properties can be more complex than in forest areas... The occurrence of higher concentrations below 6 inches is interpreted as more probably the result of physical soil disturbances as part of property development than a result of mobilization/leaching of arsenic and lead absent physical disturbance. (Glass et al., 2000)

Most contamination is found within the top 2 inches (5.08cm). However, there are some occurrences of the maximum As and Pb concentrations in individual samples being at a depth below 6 inches and is likely the result of parcel development actions and soil disturbance that altered the typical depth profiles for undisturbed locations, on a property-specific basis (Glass et al., 2000). There are several factors that will determine a property's concentration of contamination from the smelter. The King County website sums this up well in the following excerpt.

The amount of Asarco arsenic and lead is in the soil in any area of King County depends on several factors, including distance from the smelter, topography, and history of the property in question. Here are some rules of thumb to figure out the chances that your property has contamination:

Distance: The most contaminated soils tend to be in coastal King County from Seattle to Federal Way and on Vashon-Maury Island.

Topography: Soil on hillsides that face southwest tended to get more of the contamination than east and north facing hillsides because of the way the wind traveled and carried the contamination.

History of the property: Arsenic and lead tends to stay in the top six inches of soil. If the soil on a property was dug up, moved, or otherwise "disturbed" over the last 100 years, there may be less contamination there than on a property that was undisturbed for the entire time the smelter operated (from the late 1800s to 1986).

http://www.kingcounty.gov/healthservices/health/ehs/toxic/TacomaSmelterPlume/residents.aspx

Figure 3: Soil Distribution of Heavy Metal Contamination

Now that a clearer picture has been generated of the contamination distribution and what guidelines are in place for cleanup, concentrated efforts for cleanup has occurred targeting specific levels of contamination.

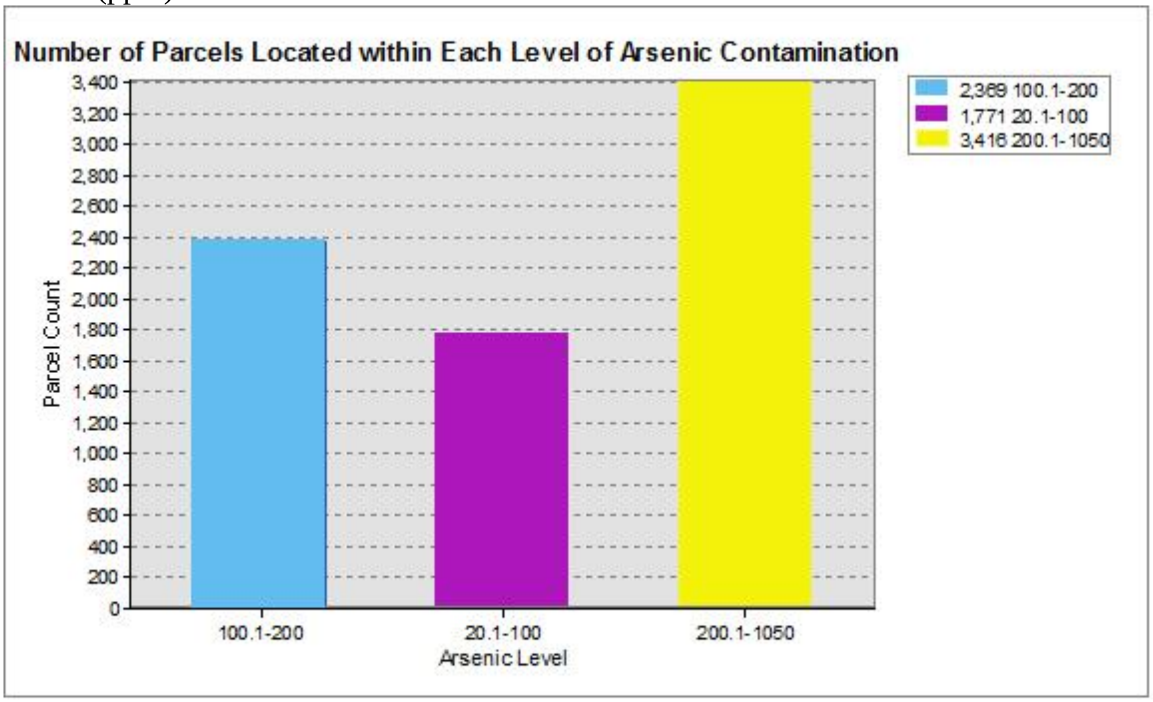
At the smelter location, cleanup consisted of building an onsite containment facility, removing the soil from the site and neighboring yards, placing the contaminated soil into that containment facility, and then terracing and capping the entire smelter site with the contaminated soil inside. The site is now monitored for leaching and other signs of contamination leaving the containment area. Information on this process can be found at http://yosemite.epa.gov/R10/cleanup.nsf/sites/asarco. All zones were children congregate, such as schools and playgrounds, with elevated contamination levels have been excavated in Tacoma, King County and on Vashon/Maury Island, but personal property has not been attended to. Many residents on the island are not even aware that there is a contamination issue. Talking to new residents on the island it is noticed that they often are not aware of the heavy metal issues. Part of the cleanup process is making sure people become informed about the heavy metal contamination on the Island.

A second settlement was reached in 2010 between ASARCO and the state of Washington. $188 million has been paid to cover the costs of the state for past ASARCO related cleanup and for funding further cleanup of highly contaminated areas. $3.9 million is going to The Washington Department of Ecology to be used for further sampling and cleanup (EPA Cleanup). Currently, one of the main programs funded from the settlement with the Department of Ecology is the Dirt Alert Soil Safety Program. The program is going to provide free testing of soils for public parks, camps, and public-multi-family housing play areas in contaminated areas as well as outreach and education on how to protect children from being exposed.

Although the Commencement Bay Superfund site does not include the surrounding areas with extreme heavy metal contamination, yard sampling is available on request. If the soils are found to be contaminated at levels afforded by the bankruptcy funds then cleanup will occur. Now, in 2011, the EPA and Department of Ecology is focusing on properties with contamination with an average above 100ppm As or any sample above 200ppm As including any sample containing above 500ppm Pb (Amy Hargrove, Personal Communications). This process consists of digging the soil up, removing it and trucking it to landfills, then replacing it with foreign soils. The soils are not remediated. Although this service has been available for contaminated sites in Ruston and Tacoma, it has not been readily available on Vashon and Maury Island.

Soil remediation is a necessity for the Vashon community in order to enable reliable, healthy and local food sourcing; an imperative for an island community that does not have easy access to produce from the mainland. Vashon residents are dealing with widespread heavy metal soil contamination from the ASARCO smelting plant. The southern half of the island was under the smelter plume windfall area, leaving it contaminated with mass amounts of the heavy metals As and Pb.

Table 2: Number of Parcels on Vashon and Maury Island within 3 ranges of As contamination. Created using Tacoma Smelter Plume Footprint Map in GIS in correlation with parcels in each contamination zone. Arsenic level is shown in parts per million(ppm).



By Shannon Clay November 2010

2,369 parcels show contamination with samples showing 100-200ppm of As. Even more concerning are the 3,416 parcels with contamination from 200ppm up to 1,050ppm. Little has been done to remediate for this contamination in this highly populated areas.

Even though ASARCO filed for bankruptcy at several of it's locations in 2005, they were bought by an international Mexico/Thai corporation conglomerate called GrupoMexico. The company operates three open pit copper mines and associated mills and copper smelter in Arizona as well as a refinery complex in Amarillo, Texas (GrupoMexico, 2011). They produce copper cathode, rod and cake precious metals and by-products (ASARCO.com/products)".

Toxicology of heavy metals

Heavy metals are defined as elements with metallic properties and an atomic number >20 (Jing et al., 2007. They occur naturally in the environment, but most heavy metals become toxic to humans, other animals and microorganisms at certain levels of contamination. Pb, however, can be a poison when ingested even in small amounts. The current standard from the Centers for Disease Control and Prevention (CDC) defines childhood Pb poisoning as a whole-blood Pb concentration equal to or greater than 10 micrograms/dL (Medical Dictionary). However, current research shows that smaller doses can affect children negatively. This is because Pb replaces other minerals that the body needs while blocking protein receptors changing the shape of the protein making it so the protein cannot connect to the metals it was originally meant to connect with. At that point, the protein cannot perform the necessary function. Depending on the person, even small amounts of Pb can cause complications. Pb accumulates in the body causing development problems in children, infertility in men, it displaces calcium in bones and in the brain diminishing brain function, and can cause low or high blood pressure (ATSDR).

> Possible complications in children include behavior or attention problems, failure at school, hearing problems, kidney damage, reduced IQ, and slowed body growth. Other symptoms of lead poisoning may include abdominal pain and cramping (usually the first sign of a high, toxic dose of lead poison), aggressive behavior, anemia, constipation, difficulty sleeping, headaches, irritability, loss of previous developmental skills (in young children), low appetite and energy, and reduced sensations. Very high levels of lead may cause vomiting, staggering walk, muscle weakness, seizures, or coma. (Medline Plus, accessed 2011)

Arsenic, the most common toxic metal widely occurring in the environment (Bhakta et al., 2010), poses serious hazardous impacts not only to human health and also has ecosystem wide consequences. It has been identified as a common cause of acute heavy metal poisoning through ingestion or inhalation posing severe health risks (Murphy et al. 1989) (Smith et al. 2009) As poisoning is caused by increased levels of As in the body and leads to apoptosis or programmed cell death (Balakumar, 2009).

King County is an area in Washington highly contaminated from the ASARCO smelter plume and has relayed the following information to the public over the internet:

> Acute (short-term) arsenic poisoning may cause nausea, vomiting, diarrhea, weakness, loss of appetite, shaking, cough and headache. Chronic (long-term) exposure may lead to a variety of symptoms including skin pigmentation, numbness, cardiovascular disease, diabetes, and vascular disease. Arsenic is also known to cause a variety of cancers including skin cancer (non-melanoma type), kidney, bladder, lung, prostate and liver cancer. (King County Health Services, 2011)

In depth information on the toxicology and health effects can be found on Agency for Toxic Substances and Disease Registry (ATSDR's) website where hundreds of research articles are documented. Most incidents of heavy metal exposure from soils occurs by ingestion of the soil, in the home from soil being carried in on garments through inhalation and through the mouth. Children, who often play in and around dirt, are often at increased risk. This shows how farming and gardening increases risk for those involved in such activities and why actions need to be made to improve these working environments.

Remediation of Heavy Metals in Soils

The most common decontamination method for polluted soils is to dig out and remove the soil and deposit it in landfills. This is a way to remove the soils from immediate concern, but does not actually remediate the issue. This process merely confines the contaminated soils with out decontamination. More rigorous remediation techniques can both remove and recover heavy metals through soil washing, scrubbing with wet screening, and through chemical methods using organic and inorganic acids, bases and salts and chelating agents. "[C]hemicals used to extract radionuclides and toxic metals include hydrochloric, nitric, phosphoric and citric acids, sodium carbonate and sodium hydroxide and the chelating agents EDTA (Salido et al. 2003) and DTPA."

(Adriano et al.) All of these methods generate secondary waste products that require additional hazardous waste treatments. Plus, in order to use these methods the soil must be transported to a treatment facility or they are tested in greenhouses and laboratories, but not enacted on site where the problem is occurring causing an increase in cost for transportation an possible spread of the toxic soils.

In situ means applying methods on site. This is beneficial for a number of reasons. In situ research lessens the chances of contamination elsewhere and helps in reestablishing healthy ecosystems within the contaminated soils providing a true remediation. Most on site treatments are done using bioremediation methods. An area of increased research, bioremediation and phytoremediation of heavy metals in soil, focus on more efficiently removing and re-accumulating the heavy metals laden within the soils rather than simply transporting the soils to a landfill. There is also research that looks at immobilizing the metals to reduce the likelihood of ingestion or metals reaching waterways (Friesl et al. 2003; Akhter et al. 1990; N.T. Basta 2000).

Originally, bioremediation defined all types of remediation using living organisms from trees to microorganisms to remove or neutralize contaminants in water and soils. At this time bioremediation often refers specifically the use of microorganisms to remediate polluted soils. In this thesis, bioremediation is used as a categorical name given to all biological remediation processes. Phytoremediation would fall within this category and is defined as "the use of plants and trees to remove or neutralize contaminants in polluted water or soil (thefreedictionary.com 2011)". This leaves no name to describe remediation using specifically microorganisms. In this paper it will simply be referred to as "remediation with microorganisms".

Bioremediation in all of its forms has been suggested as a more efficient, cost effective, and environmentally sound way to treat heavy metal soil contamination. Most research on bioremediation has been done ex-situ (in a lab/controlled environment). It is important that this research be done in-situ/in the field to make sure it is effective within the contaminated environments. If an effective method is found, many hazardous waste sites could be remediated around the country and the world. "Phytoremediation has the potential to clean an estimated 30,000 contaminated waste sites throughout the US according to the EPA's Comprehensive Environmental Response Compensation Liability Information System (CERCLIS) (Henry, 2000)."

Remediation of heavy metal contamination in soils is more difficult than other pollutants as explained previously. It is costly to dig up and haul the soils away.

> Until now, methods used for [heavy metal] remediation such as excavation and land fill, thermal treatment, acid leaching and electroreclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions, and so on. Thus, the development of phytoremediation strategies for heavy metals contaminated soils is necessary (Chaney et al., 2000; Cheng et al., 2002; Lasat, 2002)." (Jing et al. 2007)

Bioremediation is often suggested as a less expensive form of remediation compared to other processes such as digging and hauling the soils away, or capping the contaminated soils with cement. "Common remediation methods include soil washing, excavation and reburial for metal-contaminated soils, and pump and treat systems for water (Glass, 1999)." All of these methods require removing the soils from their residing location.

Phytoremediation

Within the past 30 years extensive research has gone into finding species that not only thrive in toxic environments, but that can aid in the remediation of those environments. Species range from grasses, agricultural crops, and wild plants to microorganisms and mushrooms. Inspiration for the idea of Phytoextraction occurred with the discovery of a variety of wild plants, often endemic in naturally mineralized soils that contained high levels of heavy metals in their foliage. Baker (1981) suggested this was due to the plants evolving within these toxic environments to be able to tolerate previously toxic amounts of non-essential metals within their systems (Raskin et al., 1997). Phytoremediation can be conducted in many ways. Phytoaccumualtion/ Phytoextraction is the removal of metals from contaminated soils whereby the metal is extracted from the soil, and then translocated to, and concentrated in, the harvestable parts of the plants (Mejár and Bülow 2001, Chaney et al. 2000, Cunningham et al.1995). The harvestable parts can then be ashed to extract the metal resources. The incineration of the harvested phytoextraction plants and the collection of the metals for sale is termed phytomining by Anderson et al., 1999). Alternatively, other methods of phytoaccumulation either discard the other harvestable parts of the plant, or they may be ashed to reduce size and then discarded.

These metals are accumulated through the plants need for essential metals necessary for growth. While the plant is accumulating these essential metals, they can accumulate non-essential metals and metals without known biological functions. Many of these species of plants are capable of accumulating non-essential heavy metals, including As and Pb, into the plant roots, but fewer can amass the metals into the aerial/harvestable parts for the plant. The metals are mostly accumulated through the plant roots but can accumulate them from their aerial surfaces as well. Plants that are capable of achieving a shoot to root metal concentration ratio greater than 1 are known has hyperaccumulators (Salido et al., 2003; Baker, 1999). The accumulation of metals in hyperaccumulators often reaches 1–5% of the dry weight (Raskin et al., 1997).

> The amounts of metal absorbed by a plant depend on: (i) the concentrations and speciation of the metal in the soil solution; (ii) its movement from the bulk soils to the root surface; (iii) transport from the root surface into the root; and (iv) its translocation from the root to the shoot. (Wild, 1988 as quoted by Patra et al., p 203)

Many phytoaccumulators grow slowly, remain small and do not accumulate many metals due to their size. Sea purslane is a plant that can phytoaccumulate both As and Pb, but it has only a small amount of biomass. “The ideal plant for phytoextraction should grow rapidly, produce a high amount of biomass, and be able to tolerate and accumulate high concentrations of metals in shoots (Kumar et al.1995).” A successful remediation will most likely use a larger plant that will grow quickly to accumulate more metal faster accumulating a decent amount of metal for each harvest. The greater the amount of heavy metal that accumulates before each harvest the less time necessary to fully remediate a site. The time needed for remediation dependens on “the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years” (Kumar et al. 1995a; Blaylock and Huang, 2000 as cited by Narasimha, 2003).

Other forms of soil phytoremediation include phytostabilization where heavy metal tolerant plants grow on site to reduce the mobility of heavy metals by securing the soils and reducing leaching into groundwater and the polluted soils becoming airborne (Salt et al., 1995). Most of these plants remain in place and are not harvested.

Advantages and disadvantages of phytoremediation

Bioremediation practices tend to be low cost, when compared to other methods for remediation (Lloyd and Renshaw, 2005). However, many on the ground bioremediations fail due to uncontrollable variables and the need for attention over a longer period of time than removing the soils to a landfill. In the table below is a comparison of the advantages and disadvantages of the bioremediation method phytoremediation.

Table 3. Advantages of Phytoremediation.

Advantages	Disadvantages
1. Environmentally friendly, cost-effective, and aesthetically pleasing;	1. Relies on natural cycle of plants and therefore takes time and replication
2. Metals absorbed by the plants may be extracted from harvested plant biomass and then sustainably recycled;	2. Some plants absorb a lot of poisonous metals, making them a potential risk to the food chain if animals feed upon them.
3. Phytoremediation can be used to clean up a large variety of contaminants;	3. Application varies depending on pollutant and site being treated
4. May reduce the entry of contaminants into the environment by preventing their leakage into the groundwater systems.	4. Phytoremediation works best when the contamination is within reach of the plant roots
5. Actually remediates soil rather than covering it up or moving it to another location still contaminated.	5. Requires upkeep and access to ample sun and water

Original Table from Hong-Bo et al. p24 2010 with additions made to include more advantages and disadvantages.

The costs involved in phytoextraction could be more than ten times less per hectare compared to conventional soil remediation techniques as reported by Salt et al. (1995). Other environmental benefits of phytoextraction/phytoremediation include low impact and the prevention of erosion during the remediation process because of plant coverage of the soil. To remove sufficient amounts of heavy metals with this technique, plants have to be highly efficient in metal uptake and translocation into their aboveground vegetative parts so more metal can be stored within the plant. The most limiting factor however, is the ability for the plants to be able to access the metals within the soils for accumulation.

Heavy Metal Soil Characteristics

Heavy metals within soils are distributed within the soil structure binding to various components. These binding connections determine the metals' mobility and bioavailability. The nature of this association is often defined through the "speciation" of the metal. Metals found in soil environments exist in six forms: (I) water-soluble free metal ions that are very mobile; (II) carbonate complexes, still mobile but bound; (III) metal ions occupying ion exchangeable sites that are specifically adsorbed onto inorganic soil constituents, less mobile; (IV) organically bound metals; (V) compounds of oxides and hydroxides that are relatively immobile; and (VI) metals in the structure of silicate minerals, which are basically stuck (Tessier et al. 1979; Ahumuda *et al.*, 1999). Only speciated metals forms (I), (II) and some components of form (III), are readily bioavailable (Tessier et al., 1979). This poses a problem for phytoremediation because some metals will not budge within the environment depending on how they are connected and speciated within the soil ecosystem.

Arsenic

Arsenic is found in group 14 on the Periodic table meaning that in its most elemental form (not bound to anything) it has a valence of 3. "The extent/rate of bioaccumulation and sorption/desorption processes are highly influenced by chemical species specific in the plant-water-soil systems. Whereas the trivalent states of As, As(III) also called arsenite, are mobile in the reduced soil-water systems, the pentavalent, As(V), species are relatively immobile under oxidizing conditions due to the strong fixation mechanisms in soil matrices. Both species, however, have been hyperaccumulated by plants. "As(III) and As(V) as well as methylated As species have been found in plant tissues" (Hooda ed., 2010). As(III) has been found to be less common than As(V) on

Vashon Island. Out of 25 samples taken by the Department of Ecology speciation of the smelter plume contamination was found as follows:

> As (III) concentrations ranged from a minimum of 0.086 mg/Kg… dry weight (dw) to 1.93 mg/Kg. None of the As (III) concentrations exceed the MTCA SSL of 7 mg/Kg dw…for the protection of wildlife Based on EPA Method 1632, As (V) concentrations were calculated:
>
> As (V) = Total As5 – As (III)
>
> Since only small amounts of As (III) were detected in soil samples, the As (V) concentration was only slightly less than the total As concentration. The minimum As (V) concentration was 7.140 mg/Kg dw and the maximum was 282 mg/Kg
> dw. (Sloan, 2011)

Most information on As and Pb contamination are on the total recoverable metals found in the soil because this analysis is less expensive and easier to do. Thus, the contamination from the plume is not as extreme as it could be if more of the As was trivalent (+3). Other research shows that the As does not move much within the soil column and is not prone to leaching into waterways (Glass, 1999)

Lead

Lead is in the carbon group on the periodic table. Its most common formations have tetravalent (+4) or divalent (+2) bonding ability. Pb+2 is bioavailable within the human digestive tract causing it to be the toxic form of Pb (Miretzky and Fernandez-Cirelli, 2007). Its metallic nature causes it to be attracted to organic compounds within soils. Pb is extremely insoluble and generally not available for plant uptake in normal ranges of soil pH (Manceau et al., 1996). Pb has high affinity to organic matter such as Fe-Mn oxides and clays and precipitates as carbonates, hydroxides, and phosphates (McBride, 1994). Generally, Pb is trapped within soils as insoluble Pb phosphate (Hooda et al. p315). In order for an element to be absorbed or accumulated it must be bioaccessible and bioavailable. Therefore, adding phosphate to environments low in the nutrient will actually cause Pb to bind and become insoluble. Chelators are used to make Pb more bioaccessible, but in some cases using extreme chelators has resulted in Pb becoming too soluble resulting in contamination of groundwater with the Pb (Hooda et al.). Discussion on chelators is found under the chelator subchapter.

In this type of research it is vital to know the species of Pb and As being dealt with and how different amendments and applications change the speciation to be more available and mobile or not. This type of scientific analysis for speciation of metals is rather costly. One advantage is that Vashon Island's contamination is from an anthropogenic source of contamination (the smelting). Metals from anthropogenic sources often are more mobile than naturally occurring metals (Lou et al. p5 2008; Kashem et al., p248 2011). We also have previous studies that indicate there is more As (III) than (V). Other research has found the most common species of Pb found in contaminated soils form smelters is Pb^{+2} (Morin et al, 1999).

Research is now being done within the smelter plume area to look at environmental impacts other than human health to assess terrestrial ecological impacts and see what impacts the contamination has on wildlife systems. The Model Toxics Control Act (MTCA) calls for terrestrial ecological evaluation (TEE). As cited previously, The Washington State Department of Ecology conducted a TEE looking at the Tacoma smelter plume area and the Hanford Old Orchards. The document can be found on the department's website at www.ecy.wa.gov/biblio/1103006.html or you can call and request the Ecological Soil Screening Levels for Arsenic and Lead in the Tacoma Smelter Plume Footprint and Hanford Site Old Orchards document.

Chelation

Chelation is when a ligand bonds to and around a central atom with two or more bonds forming a complex molecule. This bonding occurs with a metal ion as the central atom making the metal ion unable to bond with anything else but the ligand. Different chelating ligands are attractive to different metals. The ligands are formed in such a way that makes binding to particular metals very attractive. The attraction is so that it will debond metals from other bonds. For example, Pb commonly has 2 or 4 electrons available for binding to other elements. When a chelator, having 2 or 4 bonding locations available to Pb-, is added to Pb contaminated soils it makes the Pb more mobile. The Pb is now bonded to the ligand not the soil and the ligand is only bound to the Pb ion. Thus, the Pb is freed from the other various elements and organic compounds within the soil causing the Pb to be immobile. New bonds are made with the available ligands and the Pb becomes available.

Extensive research has been done using synthetic chelates to increase plant uptake of Pb and some with As. (Mentioned before, As on Vashon Island is mostly the more immobile As(V)). Ilya Raskin, a prominent scientist in the phytoremediation field, used ethylenediaminetetraacetic acid (EDTA) to chelate Pb and As and make them more bioavailable for uptake by *B. juncea*.

> EDTA acts by complexing soluble metals present in the soil solution. As the free-metal activity decreases, the dissolution of bound metal ions begins to compensate for the shift in equilibrium. The process continues until the supply of EDTA-extractable metal is exhausted. (Raskin 1997)

The results showed an increase in Pb uptake by the Indian mustard. "…the application of 10 millimols/kg□ of EDTA to soil containing 1200 mg/kg□ Pb resulted in a 1.6% Pb accumulation in the shoots of *B. juncea"*(Raskin, 1997). In order for a plant to be considered as a hyperaccumulator it must sequester at least 1%, but with the EDTA the plants were almost sequestering up to 2% of the Pb in their roots.

Although the research provided some exciting possibilities it also presented other problems. The chelated metals provided too much mobility allowing the heavy metal contamination to move into the groundwater and severely pollute it. After this discovery, regulators would no longer issue permits for field research of synthetic chelating of Pb in the US or in Europe (Hooda et al. p.329). EDTA is a strong chelator and results in undesired effects due to the slow degradation of the compound. Other research shows EDTA prevents cell division, chlorophyll synthesis and algal biomass production (Oviedo, 2003).

It is possible that using a weaker chelator would make Pb and As available without endangering the soil health as well as the surrounding environment. Strong chelators may bond minerals too strongly as well making the mineral unavailable to plants while a weak chelating agent may not be able to bond the mineral enough to prevent it from interacting with other compounds also reducing their availability to plants as they become more entrenched within the soils to other elements.

There are natural chelators as well. Some grasses are known to chelate by producing and releasing phytosiderophores to make metals more available for plant uptake (Ma and Nomoto, 1996). Bacteria also can secrete natural biosurfactants enhancing the bioavailability of metals (Volkering et al. 1998). These grasses could be used at the Home Remedy Site in combination with the hyperaccumulators and might be a focus of future research.

Note on Genetically Engineered Phytoremediation Research

Mejár and Bülow conducted extensive phytoremediation research by introducing proteins called metalothioneins from mammals into hyperaccumulating plants. Of particular interest were the enzymes called phytochelates (PCs). Organisms respond to heavy metal stress using different defense systems, such as exclusion, compartmentalization, making complexes and the synthesis of binding proteins such as metallothioneins (MT) of which phytochelatins are grouped under (Mejár and Bülow, 2001). Plants synthesize phytochelates when their cells come into contact with toxic heavy metals (Mejár and Bülow, 2001). The PCs allow the heavy metals to move more freely throughout the plant so that it can be stored in an area of the plant that is less toxic to the plant for better plant survival. Researchers found that through genetic engineering the over expression of these enzymes increased uptake of metals into roots and shoots but did not find an increase in overall accumulation. PCs from E. coli were also used, as E. coli has one of the strongest PCs, in *B. juncea* by Zhu et al. resulting in transgenic plants that accumulate more Cadmium than wild *B. juncea* plants. Genetic engineering (GE) is an approach to manipulate plants genetically to acquire traits that are desirable functions for humans. This type of science may lead to unwanted results with GE plants cross pollinating with wild plants and changing natural evolutionary processes. Research has shown that GE plants can affect animal's reproductive processes as in the moth that was exposed to GE pollen and became sterile (Zangerl, 2001). GE plants could cross pollinate with wild varieties causing these plants to also hyperaccumulate the toxic metals. Then the plants would be toxic to any animal ingesting the plant (Snow, 2002; Global Justice Ecology Project). Due to the uncontrollable nature of these plants, the author of this thesis highly advises against their use.

Biochar

Biochar is organic matter that has been combusted in the absence of oxygen in a process known as pyrolization creating charcoal. The term biochar is modern and, in this paper, references charred organic matter that is deliberately applied to condition soils. The modern "discovery" of biochar comes from ancient soils called Terra Preta, or Amazonian Dark Earths. Soils from archeological sites throughout the Amazon contain very high levels of charred organic material. This charred carbon is thought to be a result of agricultural practices in the area with Terra Preta dating to as early as 8000 BP (Taylor, 16), improving the quality of the native soils of the time. Even though the people that made them vanished long ago, the charred material has remained a beneficial soil constituent within Terra Preta remaining the most productive soil in the Amazon to this day.

Presently, the use of biochar is focused in three main areas of interest and <u>potential</u> use:

1. The most publicized is the possibility of using biochar for carbon sequestration.[1]
2. As a soil amendment (Taylor, 18).
3. Remediation of toxins from soil and water.

This paper focuses on using biochar for its ability to absorb water within the soils, to increase cation and anion exchange capacity, and provide beneficial environments for microorganisms and plant roots. It is important to understand how biochar is made in order to identify its soil benefits. Biochar is made through pyrolysis, which is the process of combusting organic matter, called feedstock, in the absence of oxygen. This retains carbon in solid form rather than converting it into CO_2. During this process most of the volatile elements in the feedstock change to a gaseous form and escape from the carbon

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1. Burying large amounts of biochar could potentially be a way to sink and store carbon by preventing it from entering the atmosphere, and therefore slowing or reversing climate change. Of course this is depends on the way it is produced. Often a forest used to make biofuel or biochar holds more CO^2 than what would end up being preserved in the soils.

structure. These escaping gasses leave tiny holes in the carbon structure and result in a greatly increased surface area. This increased surface area is what gives biochar most of its beneficial properties.

Two main factors affect the properties of a biochar; feedstock and treatment temperature. Different feedstocks produce different biochars with different properties. Biochar made from manure will have very different properties than biochar made from walnut shells. The original feedstock contains various matrices of compositions of elements, some volatile and some not. Mineral elements in a feedstock are not volatile and are always preserved when charred. This leaves nutrients necessary for plant growth within the biochar. Other elements will volatilize and/or oxidize to varying degrees depending on treatment temperature. The feedstock will affect both the surface area of a biochar and what elements remain in the biochar in addition to carbon. For example, biochars made form wood tend to have a pH around 7.5 and Phosphorous in the level of 6.8 g/kg, Nitrogen at 10.9 g/kg and Potassium at 0.9 g/kg of biochar (Lehmann and Joseph, p69).

Treatment temperature also has a great effect on a biochar's properties. Pyrolysis begins once feedstock is heated to 350°C at which point volatile elements begin to turn to gasses. The surface area increases as the gasses erupt forth, leaving empty macro- and micropores behind. Depending on feedstock, this continues with increased treatment temperatures up to around 750°C. When a temperature of 750°C is reached the carbon structure will start to deteriorate and surface area will decrease (Brown et al. 2006). Both the type of feedstock and the temperature at which it is pyrolized will result in production of materials for different applications.

Biochar and its high surface area can have many potential benefits for both remediation and agriculture. Lehmann and Joseph's extensive textbook, Biochar for Environmental Management, includes hundreds of research documents providing a substantial understanding of biochar. The book maintains that when feedstock is going through pyrolysis micro-pores and macro-pores are formed at different heat ranges. The micro-pores can retain nutrients, contaminants and water while macro-pores provide habitat for microorganisms and roots. (Lehmann 2009, p25). Research shows that biochar holds more water than activated carbon (Pietikäinen et al. 2000 as cited in Lehmann p87) and acts like a sponge absorbing the water and the nutrients the water contains. Depending on the biochar's properties, it can have a special affinity for certain types of molecules by attracting elements with positive and negative charges to different locations within the char. This is a form of adsorption, which is when molecules and particles bind to a surface, in this case the biochar. The high surface area of biochar contributes to nutrient adsorption through charge or covalent interactions (Verheijen et al., 2010). Surface area only increases if the feedstock is pyrolized at temperatures above 500°C (Verheijen et al., 2010). This is important in remediation and will determine whether or not certain bonds will remain in the current soil structure or change when the biochar is added.

Additions of biochar have shown increase in the cation exchange capacity (CEC) and anion exchange capacity (AEC) of soils. CEC is a measurement of how well a soil can adsorp[2] and exchange cations including K+ and NH_4+ while AEC is the measurement of how well anions, such as NO_3- and PO_4^2-, can be exchanged (WSU Tree Fruit Research and Extension Center). High surface area increases CEC because the surfaces of clays and organic matter have negative charges and can bond easily to cations. Low CEC results in nutrients leaching away. Generally, the more surface area there is in a soil, the better nutrients are stored especially with smaller pore sizes. Root hairs have a CEC also and exchange an H+ for a cation nutrient. Biochar holds nutrients providing a long-term source of organic matter for plants to draw from. High CEC prevents leaching of nutrients and metals. This allows stores of nutrients to be maintained within the soils longer creating a healthier growing environment for plants.

With the increase in CEC and AEC within the soils, biochar can have a similar effect as chelating ligand bonds because it can retain and release metal ions. It allows the attractions of the metals bound within the soils to find new attractions and move throughout the soil system. However, it is also presumed that biochar adsorps[2] metals which would decrease uptake to plants unless a strong relationship is formed between the microorganisms living within the biochar and the plants growing there. When biochar is

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2. Adsorp is different than adsorb. Adsorption is when a substance attracts and holds other materials or particles to its surface (The Free Dictionary).

added to soils, microorganisms quickly move into the macropore spaces to live. There they interact forming diverse living cultures that can be supported for centuries among the diverse pore spaces. They also aid in plant uptake of nutrients by interacting with the adsorped elements and plant roots.

Research conducted by Namgay et al. showed successful uptake by plants of Pb at certain biochar concentrations added to the soil. When biochar was added at a rate of 15 g/kg to Pb polluted soils, Pb uptake was significantly increased compared with biochar applied at a rate of 5 g/kg (Namgay et al., p641 2010). In other research biochar has been used to reduce the bioavailability of heavy metals by sorbing the metals to the char while the char released nutrients for plant growth. The only metal that had an adverse reaction was Pb. Research done by Beesley *et al.* showed that the addition of biochar to multi element contaminated soils showed an increase in soluble Pb and As while all other elements decreased in solubility over time. What was also interesting about this research was an increase in As above concentrations found in the initial contaminated soil samples (Beesley et al.), p.2286). This maybe due to As becoming more bioavailable than prior to the biochar addition.

Biochars can have widely varying properties but in general they all have a high surface area (measured in meters squared per gram) created by small pores providing infrastructure for a healthy soil ecology and retaining water. These are advantages for use in both remediation and agriculture.

Microorganisms and Bioremediation

Beneficial cultures of microorganisms (microbes) are shown to improve soil microbial ecology (Sanita di Toppi and Gabbrielli, 1999). The development and use of microorganisms is a growing field of study with a wide range of possibilites in how to best use this knowledge. Different blends/cultures of microbes are currently used to increase crop yields as well as combat heavy metals. For example, As creates a variety of responses with different microbes in nature. “Depending on the species of different microorganisms, the responses [to As] could be chelation, compartmentalization, exclusion, and immobilization” (Tsai, Shen-Long 2009). Tsai working with Singh and

Chen have conducted several in depth studies on *Saccharomyces cerevisiae* in relation to As. *S. cerevisiae* has two different mechanisms to reduce As toxicity (Tsai et al., 2009).

> Arsenic uptake by *Saccharomyces cerevisiae* occurs through three different transport systems. The pentavalent arsenate, because of the similarity to phosphate, is taken up through a phosphate transporter. In addition, two transporter systems for the trivalent arsenite have been identified. (Tsai et al., pg 662)

This is one example of the complex of interactions microorganisms can have in relation to heavy metal remediation. There are so many types of microorganisms interacting within the soil and each different species has a different response to each element.

Saccharomyces cerevisiae is a fungal yeast that is selective for monovalent metal ions meaning metals that are found with only one electron available for bonding. *Saccharomyces cerevisiae* have also been found to be able to live after ingesting high amounts of As. Certain genes have been identified in *S. cerevisiae* that are related to the resistance to arsenate and arsenite (two common species of As). When these genes were removed (documented as ACR3) there were increased arsenite sensitivities and an increase in arsenite accumulation in the yeast. Therefore, in this research *S. cerevisiae* would not be beneficial in accumulating the heavy metals into itself and transporting it to the plants because genes block the accumulation of the arsenite, but might increase uptake by plants by being able to survive in soils with high As content aiding in the growth of the plants (Ghosh et al., 1999).

Microorganisms form relationships between plants and the surrounding substrates in order to exchange necessities providing the plant with the nutrients it needs while, at the same time, providing for the microorganisms. "Typically, microorganisms are responsible for providing hormones, nutrients and minerals in a useable form to the plants via the root ecology" (Woodward, p.2). When microbes are found within plant roots and plant rhizomes they are called rhizobacteria. "The presence of rhizobacteria increased concentrations of Zn (Whiting et al., 2001), Ni (Abou-Shanab et al., 2003b) and Se (de Souza et al., 1999a) in *T. caerulescens*, *A. murale* and *B. juncea*, respectively." (Jing) Here Jing is illustrating how plants necessary elements are provided for by the presence of these microorganisms

Lactobacillus is a specific species of microbes that creates lactic acid and are known as LAB. These bacteria can also live in environments containing high amounts of heavy metal contamination. “Results of metal resistant ability also implied that tested LAB can easily survive with high As and Pb containing environment “(Bhakta, 2010). The Lactic Acid bacteria mentioned here were found to be specifically *Pediococcus dextrinicus* and *Pediococcus acidilactici* which were not used in this study, but this information was released after the application of the BioKlean$^{TM®}$ the microorganism blend used for this study. LAB are very common in the soil/plant realm. “[L]actobacillia are ubiquitous, present on the surface of all living things and especially numerous on leaves and roots of plants growing in or near the ground (Fallon, p 89)”. They aid in plant growth and help maintain plant health.

As discussed previously in the biochar section, pore size and distribution are important factors within soils in order to maintain healthy ecosystems for microbes also determining the amount of water and air content important for life (Murányi, p3). Conditions within the soils are very important for successful microbrial inoculation. In polluted environments, root systems may be restricted due to low nutrient content and availability decreasing the success of the microbial inoculation. By introducing microorganisms into a polluted environment, root growth will likely increase and create a healthier environment for plants. “An efficient microbial inoculum generally increase the vitality of the soil / plant interface (Murányi, p4)”. Due to the need for a successful inoculation that can withstand high levels of As and Pb, a particular microorganism brew/culture was selected for this research.

With this background on smelting pollution and phytoremediation the goals of this study are better understood. Bioremediation is very site specific and greatly depends on the soils present. The next chapter will provide more in depth information on bioremediation specific to the Vashon Island site.

CHAPTER II Bioremediation Specific to Vashon Island

The Home Remedy Study Site

The study site was provided by resident Amy Wolf who lives on 2 acres on the southern end of Vashon Island. Two accessible areas on the property were used. One was a grassy side yard used previously for gardening, called the *grassy area*, where all the trees had been cut down in the early 60s. The other location is located in the back yard and will be referred to as the "previously forested area." This is a newly cleared area prepared a few months prior to the onset of the project. Both areas contained concentrations of Pb and As above Washington State and EPA allowable limits. These initial values were obtained onsite using a Niton XRF field analysis instrument with an environmental toolkit.[3] These values were confirmed later using an in lab method (See Chapter 4, Table 4).

Figure 5. Map of Vashon Island Site Location

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3. This analysis was conducted by myself (Shannon Clay) and Andrew Long, a project constituent.

The grassy area was covered in *Dactylis glomerata* (orchard) and *Festuca sp.* (fescue) grasses. The underlying soil is a humic sandy loam with hydric areas. A visible horizon appears during the rainy season (winter), which seems to delimit soil-gas penetration by means of a fluctuating water table. Other spots within this same area had more of a clay composition with a sandy loam top soil with hydric limits. The soil pH ranged between 6-6.5. The previously forested had *Psuedotsuega menzesii* (douglas fir*), Alnus rubra* (red alder), with an understory association of *Pteridophyta* including *Pteris munitum* (western sword fern) *Pteris aquiline (*common bracken fern), *Vaccinium sp*., *Gaultheria. shallon* (salal) and *Ribes sanguineum* (flowering red currant). The soil in this location has a 1inch deep, of humus and detritus. The soil is a loamy sand. pH varies slightly, averaging 6. Over the last 5 years rain fall in this area averaged around 46 inches annually[4]. The trees all around the site are 15m(50ft) –27m(90ft) tall. Insolation is more limited in the spring and fall by canopy height. Summer sun is ample because the location of trees does not shade those areas.

Restoration at this site is aimed to test practical methods for bioremediaton of As and Pb in soils. After researching possible plants and soil amendments three applications were chosen. These were applied together and separately to determined individual impacts and possible synergistic effects. *Brassica juncea*, brown mustard, was chosen for the plant, a microorganism blend called BioKleanTM as well as a soil amendment called biochar were chosen to aid in sequestration of As and Pb by *B. juncea*.

Phytoremediation with *Brassica juncea*

There are many candidates for phytoremediation of this site including Sunflowers, sea purslane, and ferns. Sunflowers (*Helianthus annuus*) may have been a better plant to use in an area that has more sunlight than this study site. However, in some research sunflowers, did not accumulate as much As as *Brassica juncea* (Scholtz, 2006). *Pteris vittata,* common name Chinese brake fern, is a well known accumulator of As but is not a Pb accumulator. It could be possible to use the fern in a rotation with another plant that accumulates Pb. More information about *P. vittata* can be found in Chapter IV

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4. Tahlequah rain gauge data from King County precipitation data

Furthering the Research. Hyperaccumulators, such as sea purslane, grow slowly especially when contamination is high (Jing et al.) Sea purslane is a small plant and in soils that have more sand than the soils on Vashon Island. Many plantings and harvesting would be needed for sea purslane because of its size.

Brassica juncea (Brown Indian Mustard) was chosen for a number of reasons. Particular emphasis has been placed on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species because of their relation to wild metal-accumulating mustards (Raskin et al. citing Kumar et al., 1997). Even though *B. juncea* has a lower metal accumulating capacity than sea purslane for example, it has higher growth rates. These growth rates may allow for more biomass to be harvested and an overall greater amount of the contaminants (Kumar et al.,1997).

For the remediation of the heavy metals As and Pb, *B. juncea* has been found to remediate both through hyperaccumulation and phytoextraction successfully (Salido, 2003, Kumar et al. 1995). One article found Pb to be accumulated around concentration levels of 25mg/g of Pb per plant in the roots alone (Clement et al., p50 2005). Cultivar 426308 of *B. juncea* was found to be the best Pb accumulator by Kumar et al. while cultivar 211000 was found to be a good accumulator for As by Scholtz during his thesis work on Uranium contaminated soils. These and other *B. juncea* seeds were requested from the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS part of the Germplasm Resource Information Network GRIN). These specific species had research finding these species to accumulate As and Pb. However, in order to maintain the collections NPGS only allows 200 seeds of any variety to each individual request. This amount of seed would require an extra year to prepare for the study in order to grow these plants to harvest there seeds. At least 1200 seeds are needed for this study. More information on these seed cultivars can be found in the Appendix.

An available varietal species of *Brassica juncea* was chosen for the research from FEDCO seeds. 3232PO Pung Pop Mustard Gene Pool OG (40 days) was chosen because it was known to grow large leaves and thick stalks in a short period of time. This would likely lead to amassing more As and Pb. The varieties' description reads as follows:

> Open-pollinated. *Brassica juncea* Pung Pop is an acronym for Pungent Population, a gene pool Morton selected out of breeding populations from Miike-Horned crosses, pungent Indian mustards. The results are rapid-

> growing large plants with thick stems, big dark green leaves and handsome red veining...Survived Roberta's overwintering trial. OT-certified. (FEDCO)

In addition to being rapid and large growing, this variety was also hearty enough to overwinter providing more accumulation time. Any quantity could be obtained by any one allowing this variety to be easily accessed by other's who would like to do remediation in their yards.

A major issue with using *B. juncea* realized through *in situ* studies was that the shoot metal accumulation in plants grown hydroponically as well as in lab controlled conditions accumulated much more contaminants than *B. juncea* grown in the field. This phenomenon is explained by the low bioavailability of heavy metals in soils (Raskin, 1997). In order to make the metals more bioavailable, chelation can be used. Illya Raskin headed numerous research studies using chelation to enhance Pb uptake by *B. juncea.*

> Vegetation growing in heavily contaminated areas often has less than 50 mg g□1 Pb in shoots [due to metals being binded to the soil matrix][Cunningham et al. 1995]. Even plants that have a genetic capacity to accumulate Pb (e.g. *B. juncea*) will not contain much Pb in roots or shoots if cultivated in Pb-contaminated soil. The solution to the metal availability problem came with the discovery that certain soil-applied chelating agents greatly increase the translocation of heavy metals, including Pb, from soil into the shoots [Blaylock et al.] (Illya Raskin, 1997)

The application of chelators may be the key to successful phytoremediation for heavy metals. Some metals are adsorbed to oxides, but these bonds can be broken releasing the metals through natural and applied chelates, or the increase of cation and anion exchange rates. The soil composition will determine the soil ecosystems ability to allow movements within the soil. The amount of clay and organic matter, as well as the texture and cation exchange capacity (CEC) within the soil will influence the migration of the metals (Mason, 1992).

Application of Biochar at the Home Remedy Site

In using biochar at the Home Remedy Site, it was not clear how the biochar would interact with the mustard plants, microorganisms, and soil in metal accumulation within the plants. The intended purpose of using the biochar was as a soil amendment to increase cation and anion exchange and water retention for the mustards while providing nutrients for the plants as well. This would aid the plants by providing necessary nutrients, lengthening their root systems and being able to access more of the heavy metals. It was also theorized that it would benefit the microorganisms being applied in some of the same plots. The proposition used in biochar application for this study is that the biochar will aid in the metals movement toward the plant through microorganism plant relationships occurring around and within the biochar.

An apple tree (*malus sp*.) was the feedstock pyrolyzed for the research. This is a good biomass source for the Pacific Northwest because they are common and because the sapling branches often are trimmed yearly providing a locally abundant source of feedstock for making biochar. Britton Shepard, founder of Renata Biochar Systems, provided access to his kiln in Fall City, WA to produce our biochar. The kiln is styled as a 2 door steel retort. The feedstock was pyrolized at temperatures between 500-700° C using chopped wood as the fire and heat source. On the removal of the biochar from the kiln it was immediately dipped into a barrel of water to prevent further burning and to reduce the heat to a manageable level. The biochar was then pulverized into smaller pieces using a grinding machine. It was distributed into the plots by tilling at a rate of 20tons/hectaracre equaling 1.5 gallons per plot.

Biochar has been shown to make acidic soils more alkaline when added unless the biochar is acidic and then it will actually lower pH (Lehman, p. 262). This was a concern for this research because Pb has been found to be more extractable in acidic soils (Rieuwert et al. 2006) as well as more mobile and sequesterable (Matos et al., 2001, Udon et al., 2004) (Kashem et al.). As availability has a strong and negative correlation with pH as well (Luo et al., p5 2008). However, it has been found that inorganic As can be relatively mobile in soils in both oxidation states, particularly in alkaline soils (Hooda, p142, 2010). The Home Remedy Site soil is slightly acidic with a consistent pH of 6 when tested with pH strips.

It was hypothesized the biochar would increase the ability for cation exchange increasing the mobility within the soil and cause the As and Pb to be more available for accumulation in the plants. This would occur because of biochar's surface sorption abilities breaking soil bonds and forming new bonds within the biochar. When the bonds are breaking in the soil the metals may become more available during that time. Also, with the addition of microorganisms that can form chelates around heavy metals, biochar can react well within these chelated environments sorbing the chelates (Lehman, p.263). Then the Pb would be available to certain microorganisms that may transport it to the mustard plant roots. Even though the concentrations of extractable As and Pb are likely to increase from adding biochar, the amount accumulated by the plants might decrease because of the affinity of the heavy metals to the surface of the biochar (Namgay, Abstract 2010). Biochar will likely attract the heavy metals within the soils to its surface and could result in a decrease in available Pb and As because it is bound to the biochar. It is hypothesized that microorganisms could facilitate uptake of these non-bioavailable metals into plant tissues while biochar makes the metals in the soils less bioavailable and less toxic but still provide an environment where these metals could be permanently removed by plants.

Application of Microorganisms at the Site : SCD BioKlean^{TM}

BioKlean^{TM} was chosen for use to aid the growth of the mustard plants and in the accumulation of metals. This brew is made to support itself with bacteria that provide nutrients and necessities needed for the other bacteria within the brew.

> The SCD consortium are ecosystems consisting of balanced populations of different probiotic strains trained to live together via growth selection, which in turn translates into balanced metabolic changes of the application environment. Conceptually, the yeast have the ability to assimilate glucose as a substrate and produce pyruvic acid through metabolism of the saccharide decomposed system. Pyruvic acid can be used as a substrate of facultative anaerobic lactic acid bacteria. In this way, if the lactic acid bacteria using the metabolite of yeast multiply, the formed lactic acid becomes the substrate of photosynthetic bacteria and they can be multiplied. Then yeast uses the saccharides formed by this photosynthetic bacteria as a substrate and can multiply repeatedly. This implicates that the microbes in SCD Probiotics Technology continue to aid each other to keep alive and stay strong in the environment. The lactic acid bacteria (LAB) produce lactic acid as the major metabolic end product of carbohydrate fermentation. LAB are also characterized by an increased tolerance to a lower pH range. This enables LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production. Through the metabolism of LAB, CO2 (carbon dioxide) is formed. This is used by other

> species in the consortia as a source of energy to their own metabolic systems, e.g. phototrophic bacteria. These cultures synergistically work to inhibit the growth of pathogenic harmful bacteria through competitive exclusion. In addition, the product contains metabolites produced by the consortia of beneficial microbes and the chemical characteristics of these metabolites contribute to antimicrobial properties, neutralization of toxic substances as well as contribute to health benefits.

For this feasibility study, this culture/blend of microbes were applied in order to aid plant growth, transport of the arsenic and lead, as well as create relationships between the plants, soils and biochar. The microbial brew chosen for this research was easily accessible at reduced costs already prepared and it contains a few microorganisms shown to aid in the remediation of heavy metals while benefiting to the plant growing process. The microbial content is based off of the microblends called EM (Effective Microorganisms) discovered in Japan in the 1970's. This specific culture has been shown to increase agricultural yields of a variety of crops especially when used in combination with other soil amendments (Hussain et al., 1999). Studies also identified greater resistance to water stress (Xu, 2000) and a better penetration of roots (Ho In Ho and Ji Hwan, 2001). The effect of EM on test white mustard (*Sinapis alba)* seeds was studied in a germination experiment of four days. EM vitalized the germination, because the average shoot length was increased by 25% (muryani, p. 10). This would be helpful in the faster accumulation of heavy metals. EM helps to re-establish a balanced soil ecology and to combat oxidative corrosion in plants and humans (Deiana, Dessi, Ke, Liang, Higa, Gilore, Jen, Rehan, Aruom, 2002)

SCD BioKlean™ is a specialized EM culture of microorganisms used more specifically for cleaning a wide variety of surfaces and contaminants, but it is also greatly used for the treatment of polluted water and solid waste. It has been created by SCD (Sustainable Community Development) along with other probiotic brews to aid in the responsible care of ecosystems. Mainly, for this research the use of BioKlean™ is aimed at aiding in plant growth in heavy metal contaminated soils. Following is the list of microorganisms in SCD BioKlean™:

Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus *fermentum, Lactobacillus. plantarum, Lactococcus lactis, and Streptococcus thermophilus, Saccharomyces cerevisiae, Bacillus subtilis, Rhodopseudomonas palustris and Rhodopseudomonas sphaeroides*

Below is more information about each type of microorganism in the brew to help explain how each can contribute to the bioremediaton process.

Bifidobacterium spp.

Bifidobacterium sp: human gut microflora that control pH by producing lactic and acetic acids. Also helps to control pH of soils.

Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium longum

Lactic acid bacteria (LAB)

Lactobacillus spp. Are used as the general substrate for growing the other microorganisms in the culture. They are acid tolerant bacteria that all produced acetic acid and lactic acid during fermentation and inhibit yeast growth while improving aerobic stability. "Lactate fermentation facilitator as being obligate heterofermenter"(Microbial Consortium Mechanism) These bacteria are known for their ability to make ATP from sugar and milk.

Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus fermentum, Lactococcus lactis, Streptococcus thermophilus

Yeast

Saccharoyces cervisae; as discussed earlier this yeast has a particular ability to transport As without dying. It's role within the microorganism brew is creating movement and aeration while also providing food for other microbes.

Gram-positive spore forming bacteria

Bacillus subtilis var-anti fungal and the phototrophic bacterium that produces proteolytic enzymes. These enzymes break down proteins into amino acids.

Probiotic benefits and co-growth promoting the mucus layer secretion by other microbial strains and production of beneficial enzymes.

Photosynthetic bacteria

These help to create biomass from sunlight to feed other microorganisms.

Rhodopseudomonas palustris- phototropic bacteria that converts sunlight to cellular activity and converts CO2 to biomass and fixes nitrogen (*R. palustris* citation*)* It is found in soils and water and makes its living by converting sunlight to cellular energy and by absorbing atmospheric carbon dioxide and converting it to biomass. This microbe can also degrade and recycle a variety of aromatic compounds that comprise lignin, the main constituent of wood and the second most abundant polymer on earth. It grows both in the absence and presence of oxygen.

R. sphaeroides- phototropic- fixes nitrogen

SCD Probiotics products activity can consume and also mobilize nutrients. When former researchers have measured the chemical composition of the solution phase by ICP, The concentration of macroelements (Ca, Mg, Na, K) did not change significantly from before to after inoculation. However, Phosphorous and Manganese decreased.

> …the original P concentration (34 mg/L) decreased down to 17 mg/L, what was caused by the P consumption of growing microorganisms. The Mn concentration decreased down to a minimum value then started to increase back again. The Cu concentration decreased continuously, indicating that this element was needed for microbial growth (Muryani p7).

SCD Probiotics products have shown to be highly efficient increasing biological activity within soils and maintaining the level of activity for long periods of time.

SCD Probiotics are also more acidic and causes decrease in soil pH during microorganism activation. This usually occurs by a factor of .9pH units (Muryani, p. 3). Acidic soils are better for Pb and As movement and phytoaccumulation (Luo et al., 2008; Bienfait et al., 1986)

BioKlean™ and other SCD Probiotic brews provide a controlled environment preventing molds and other harmful bacterias that cause plant and animal diseases. Working together, the microorganisms within the brew keep each other alive while keeping non-beneficial microbes away.

> Antimicrobial capacity of SCD Probiotics products has been evaluated and challenged with a large variety of unpleasant microorganisms, including bacteria (e.g. Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and Staphylococcus aureus), yeast (e.g. Candida albicans), and mold (e.g. Aspergillus niger, and Fusarium oxysporum). Accordingly, the outcomes from the antimicrobial testing confirm that SCD Probiotics products are capable of controlling and reducing the growth of unfriendly microorganisms efficiently. (SCD Probiotics, 2008)

SCD Probiotics, in collaboration with other labs, show the ability for their microorganism brews to fight off these unwanted bacteria and fungus. *Pseudomonas aeruginosa* is commonly found in soil, water, human skins, and medical devices. It is a typical cause of infections in pulmonary tract, urinary tract, burns, wounds, external ears and also blood. Aspergillus niger is a fungus, usually causing black mold disease on fruits and vegetables (e.g. grapes, peanuts, and onions). In addition, it is a general cause of fungal ear infections in human. Fusarium oxysporum is a fungus, usually causing Fusarium wilt disease (Panama disease or Agent Green) in plants. SCD Probiotic brews prevent these unwanted funguses and molds creating a safer growing environment for plants and other bacteria and a safe working environment for humans. This means it can safely be used in the global environment.

Research Questions and Hypothesis

With these three bioremediation applications; *Brassica juncea*, Biochar and BioKlean™, the Home Remedy Project Feasibility Study will examine the ability for the amendments to work together in order to help the *B. juncea* grow well for phytoremediation. The microbial brew will aid the plant in its growth and help assimilate the Pb and As contaminants as the biochar is chelating the minerals slightly by increasing cation exchange, providing nutrients to the mustard plants, and providing substrate for the microorganisms.

This thesis will address the following hypotheses. The main hypothesis being tested in this feasibility study is that soils will show a significant decrease in heavy metal concentrations of As and Pb from the bioremediation treatments. Another hypothesis being tested is that the guild with all three elements working together will result in the greatest decrease in Pb and As concentrations.

CHAPTER III Materials and Methods

This chapter outlines and explains the materials needed for the methods chosen to perform the feasibility study in situ over the spring and summer of 2011.

Overview

With the assistance of volunteers interested in the study, the soils were prepared for the experiment by tilling. These individuals also assisted in monitoring the site over the growing season. The general experimental approach was to apply various As and Pb bioremediation treatments and determine after one growing season if they decreased metal concentrations in the soils.

The following is a list of the materials and a description of the methods used in the feasibility study.

Materials

XRF Analyzer	Random number generator	Gloves specific to site
String	pH strips	Log book
Stakes	Large stakes for fencing	Dry storage area
Broad fork	Deer fence	12 cup glass measurer
Plastic spoons	Shovel	BioKlean^{TM}
Measuring tape	Dionized Water	4 rain barrels
Cardboard bowls	irrigation lines	Water source
4 oz glass jars	Attachments for drip line to barrel	
Drip nozzles	Rain gauge	

Experimental Design

To select the sites for the experimental plots, the most contaminated locations within the study site were located. A Thermo Scientific Niton XRF Analyzer with an environmental analytical tool was used to obtain As and Pb measurements in the soils. The locations chosen for test plots contained 30ppm-200ppm As and 0 ppm-548 ppm Pb. The highest concentrations were found on recently cleared forestlands within the study site. Sixteen one square meter plots plus two control plots were defined using string and stakes within the highly contaminated areas. Eight plots were located in the previously forested area and another eight were located in the already cleared grassy area. There was one control plot in each area, one for the previously forested area and one for the grassy area. Then the sod was removed and the plots were tilled down to seven inches using a broad fork. Only seven inches were tilled because the metal contamination decreases between 6 inches (15.24cm) down to 12 inches (30.48cm) and there is no contamination usually found below 12 inches (Glass et al., 2000). Soil samples were then collected for the initial soil heavy metal concentration after the soil was homogenized/mixed by tilling.

At each plot, soil samples were taken randomly at each depth. Plastic spoons were use to collect the soil with a new one used for each sample. Each 1 m square was divided into a 16 square grid using a piece of string. A random number generator was used to determine which square within the grid was sampled from without repeating the same square for the same depth. Three samples were taken randomly at 2 inches deep and three samples were taken from 6 inches deep within each plot. The three random samples were mixed together in a cardboard bowl (one bowl for each depth) and then 4 ounces were sub sampled into a glass jar for the subsequent analysis in a professional lab. This was repeated for each depth taking three samples at 2 inches, combining in bowl, and three samples at 6 inches, combined in own bowl, for all 16 plots and the two control plots producing two samples for each plot for a total of 36 samples.

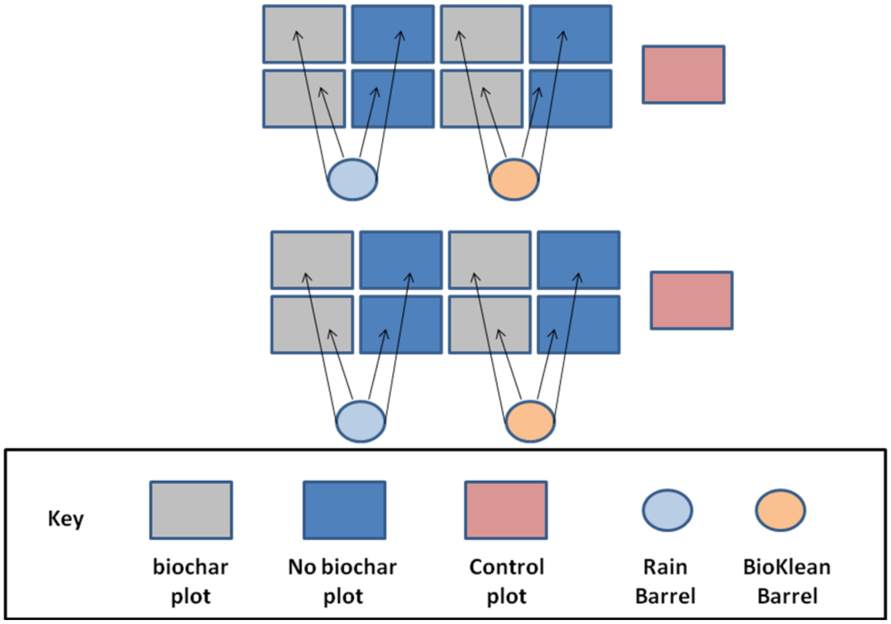
The samples were analyzed by Freidman and Bruya, Inc. in Seattle, WA. Friedman and Bruya is a company that conducts analysis of organic and inorganic compounds using federal and state approved methods. They are accredited by the National Environmental Laboratory Accreditation Program for metals testing, the Washington State Department of Ecology, Oregon Department of Environmental Quality, and the California Department of Health. At the lab the samples were further homogenized, while removing larger particles with a .10 mm screen. Each soil sample was chemically digested using EPA method 200.8/6020 to be prepared for analysis with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Due to a miscommunication with Friedman and Bruye total As and Pb concentrations were not measured using the total metals method 200.8 as desired. Thus, only recoverable, not total, metals were measured. The consequence of not having the total metals within the soils is that there is no way to know if metals decreased because they became insoluble, but remained in the soils.

Experimental Treatments

The three different amendments (mustard plants, Biochar and BioKlean^{TM}), in different combinations were distributed amongst the plots. One gallon of biochar was poured, using a gallon bucket, and then tilled into eight plots, four in the grassy area and four in the previously forested area. Then Indian Mustard, *Brassicae juncea* Pung Po Pop Mustard seeds were planted in every plot (except the two control plots) in rows 1.5 feet apart and each seed 1" apart and 1" deep. This allowed three rows of plants to fit in each plot. One week later a drip irrigation system was installed and eight plots were inoculated with the SCD BioKlean^{TM}. Microorganisms were applied weekly at a rate of 450 ml per plot every week until harvest of the mustards (around 10-14 weeks). The microbes were diluted and activated by putting 1:50 parts water in two of the four 55 gallon barrels. Each barrel was connected to four plots and once a week 7.6 cups SCD Bioklean^{TM} were diluted with 23.75 gallons of water. This approach delivered water or the inoculant to the 4 plots connected to the barrel at a rate of two gallons/hour for a total of 450mL BioKlean^{TM} per plot per week. The two barrels without microorganisms delivered only water. A mark was made on each barrel to represent the 23.75 gallon line after the first inoculation to maintain proper measurement.

The research plots were organized into groups of four. Two plots in each set contained biochar and mustards, while the other two contained only mustards. Each set of four plots was watered by a rain barrel with two of the barrels delivering the BioKleanTM microbrew. Thus, a total of four plots contain BioKleanTM, Biochar and Mustards, four contain BioKleanTM and Mustards, four are treated with Biochar and mustards, and four have only mustards.

Figure 5. Distribution of Amendments



This chart diagrams the distribution of amendments to the plots and what each plot consisted of.

These plots were monitored while the two control plots were sampled every 6 weeks to determine the natural variation in the soil metal concentration over time after being tilled. A deer fence was erected around both locations to prevent animals from eating mustards and interfering with the plots.

When the rain barrels containing BioKlean™ were inoculating the soils, the other two rain barrels were delivering the same amount of water to the un-inoculated plots. Watering was monitored by the residents to ensure plots were being watered weekly. During dry times, a protocol was followed to provide extra water for the mustards. To make sure each plot received the same amount of water, the rate at which the water left the hose was kept equivalent throughout the watering process by watering each plot for 5 seconds and turning the hose on to maximum. Watering occurred only in the morning or in the evening to avoid mid day evaporation. A water resistant journal was kept on site so all participants could note observations and activities at the site. Rain meters were placed at both of the sites to monitor average rainfall.

The mustards were thinned two weeks after sprouting. The plants were monitored until they began to bolt. Harvesting the plants occurred when they bolted but before they began to grow seeds. All of the plant matter, roots, shoots and flowers were kept and stored in a refrigerator at 4°C. The plants were dried and tested for the amount of Pb and As accumulated in the roots, and above ground biomass. This testing was conducted using the Niton XRF. A final sampling of the soil was collected , using the same transect sampling methodology mentioned prior, after 2-3 rounds of plants are harvested before the first winter freeze. All of the soil samples were sent to Freidman & Bruye. All the sampling procedures appear in the appendices while the 200.8 EPA method can be found online using Google search. The sample test results were then compared for before and after concentrations using a both a paired t-test and the Wilcoxin Signed Rank Sum Test to test for significant differences.

CHAPTER IV Results and Discussion

This chapter provides the results of the study followed by a discussion on the obtainable results.

Observations of mustard plants over the growing season

Seedlings sprouted two weeks after being planted. They grew slowly and did not start producing adult leaves for another 2 weeks. Three and a half days later over half of the plants were missing with little sign that plants ever grew in half of the plots. In a couple of the plots short, one or two broken stems could be seen barely rising above the ground. It is possible that these broken stems are evidence of animals eating the plants. Often plants that are weaker or not healthy are eaten by insects or other grazers. Even more interesting was the realization that the only surviving plants were in plots containing biochar. All plots containing biochar still had living mustards except for one (1F4). Table 4 displays the number of mustards surviving in each biochar plot. This suggests that the mustards would have not survived without the biochar. More mustards were growing in the previously forested plots than in the grassy area likely due to the clay soils in the grassy area. The mustards did not flower for another 6 weeks and some not until 7-8 weeks. They grew to be on average 54.86 cm (1.8 feet) tall including the flowering body.

Table 4. Number of surviving mustards per plot (only plots with biochar)

Previously Forested Plots	# of Mustards	Grassy Area Plots	# of Mustards
2F1	51	1F1	2
2F2	48	1F4	0
2F7	9	1F7	21
2F8	32	1F8	10

After the first living plants were harvested in weeks 7 and 8, a second round of mustards were planted. The same die off happened when the first mature leaves were beginning to form leaving only living mustards within the plots containing biochar (excluding 1F4, an all clay soil) supporting the idea that the mustard survival was due to the biochar. Mustards did not grow large leaves or grow quickly in this study. This may be due to the inclement weather patterns for that growing season. Due to the slow growth of the mustards only 2 rounds of mustards were planted before the first frost.

Soil Sample Analysis Results

Initial soil samples were collected May 25th, 2011 and after treatment soil samples were collected September 24th, 2011. The results of the soil sample analysis for As and Pb concentrations appears in Table 5.

Table 5. Soil Samples of Arsenic and Lead Concentrations in ppm

Plot	Initial 2in As	After 2inAs	Initial 6in As	After 6inAs	Initial 2inPb	After 2inPb	Initial 6in Pb	After 6inPb	Treatment
1F1	58.6	64.2	68.0	72.3	78.9	70.8	120	75.3	B, K, M
1F2	41.4	71.2	31.5	48.9	81.7	104	47.1	60.5	K, M
1F3	75.5	58.5	57.1	81.5	103	101	69.2	80.5	K, M
1F4	73.3	64.9	72.1	33.3	131	42.8	106	25.8	B, K, M
1F5	47.1	69.1	29.0	54.2	60.2	113	44.9	81.0	M
1F6	51.0	36.5	51.6	31.2	86.9	46.2	101	41.8	M
1F7	34.3	49.1	41.2	92.6	55.7	58.3	61.5	85.6	B, M
1F8	45.9	35.6	91.2	43.6	91.3	50.2	180	66.2	B, M
1FC	44.2	37.6	29.1	32.5	65.9	51.2	47.9	48.5	Control
2F1	125	119	135	107	477	223	156	148	B, M
2F2	170	72.5	125	111	552	113	169	136	B, M
2F3	120	86.0	79	81.7	187	172	92.8	166	M
2F4	72.3	126	134	56.0	167	219	296	67.9	M
2F5	104	112	97.1	65.8	162	213	107	86.1	K, M
2F6	156	117	103	54.7	214	172	106	83.4	K, M
2F7	105	100	77.6	54.8	313	193	145	63.4	B, K, M
2F8	48.2	106	68.7	67.0	75.9	159	117	108	B, K, M
2FC	125	69.4	107	78.8	180	155	182	65.6	Control

B=Biochar K=BioKlean™ M=Mustards [green box] = contained living plants
This table displays not only the heterogeneity of the soils, but also the patterns of increase and decrease of As and Pb within the sixteen test plots and the control plots.

Variability of Pb and As

Overall, As and Pb soil concentrations showed large variability before (As = 81.33mean ± 37.7 standard dev. Pb=145.56 mean ± 111.20 standard dev.) and after ((As = 71.13 mean ± 27.33 standard dev. Pb= 104.06 ± 55.98 standard deviation) treatment indicating large heterogeneity in the soil composition. There was also significant variation in the direction of the change in metal concentration over time. Both increases and decreases in As and Pb concentrations were observed over the study period. Increases of metal concentration were unexpected and suggest processes transporting metals into the site, such as movement by insects or complex processes like microorganisms unbinding metals allowing for their transport. Because only recoverable metals were measured, it is also possible that microorganisms changed the metals to become mobile or immobile where the total concentration is not actually changing but simply becoming unavailable for counting within the lab process used by Friedman and Bruya, Inc. because the metals are now insoluble.

Differences Before-After Treatment

The main hypothesis being tested was that there would be a significant decrease in As and Pb concentrations within the soils following the treatments. Only plots treated with Biochar+mustard and Biochar+ BioKlean™ +mustard showed a significant decrease in Pb when using a(p= 0.013, paired t-test). There were no significant decreases in As concentrations. Due to the small sample size and the possibility that the data was not normally distributed, the nonparametric Wilcoxon Signed Rank Sum Test was also conducted to test for significant differences. The results confirmed that there was a significant decrease in Pb in plots treated with Biochar+ BioKlean™ +Mustards (p= 0.006 and with Biochar+Mustard (p= 0.025).

Control Plots Over Time

The control plots also showed high variability of As and Pb over time (Table 6). They showed an overall slight decrease overtime in concentrations of both As and Pb. The control plot in the grassy garden area stayed relatively the same for both As and Pb whereas the control plot in the previously forested area decreased in total Pb and As concentration, however, this decrease was not statistically significant. Figures 4 and 5 illustrate these changes over time using a line graph. It can be seen that the previously forested area has an initial

Table 6. Time Series Control Plot Results

Control 1F	**Early June**	**July**	**August**	**Late September**
As @ 2”	44.2	35.0	33.0	37.6
As @ 6”	29.1	33.4	36.0	32.5
Pb @ 2”	65.9	50.1	45.0	51.2
Pb @ 6”	47.9	50.6	44.0	48.5
Control 2F	**Early June**	**July**	**August**	**Late September**
As @ 2”	125	102	93.0	69.4
As @ 6”	107	84.6	95.0	78.8
Pb @ 2”	180	116	89.0	115
Pb @ 6”	182	42.6	75.0	65.6

higher concentration of both As and Pb. After the controls are tilled both drop in concentrations of As and Pb while the previously forested area decreases more than the garden grassy area but still remains with higher concentrations by the end of the season.

Figure 6.

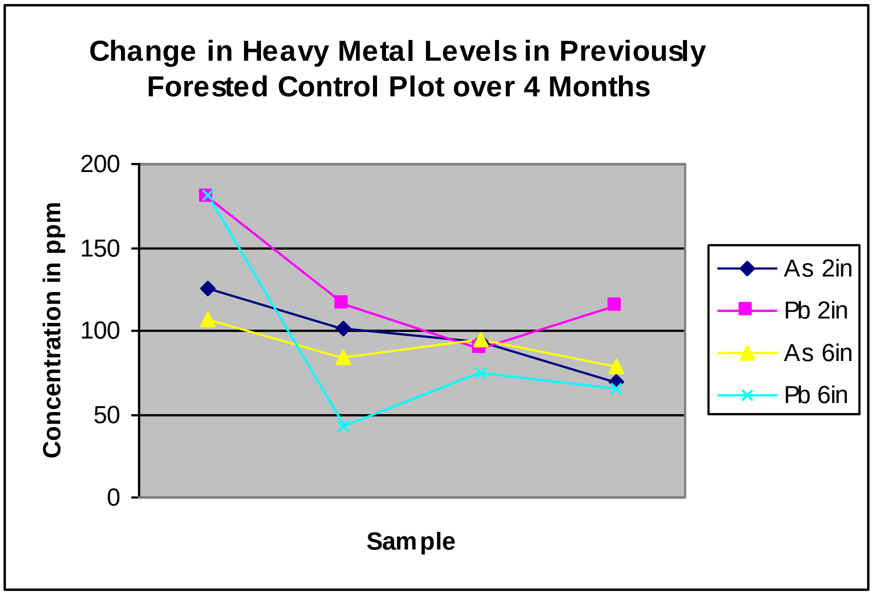
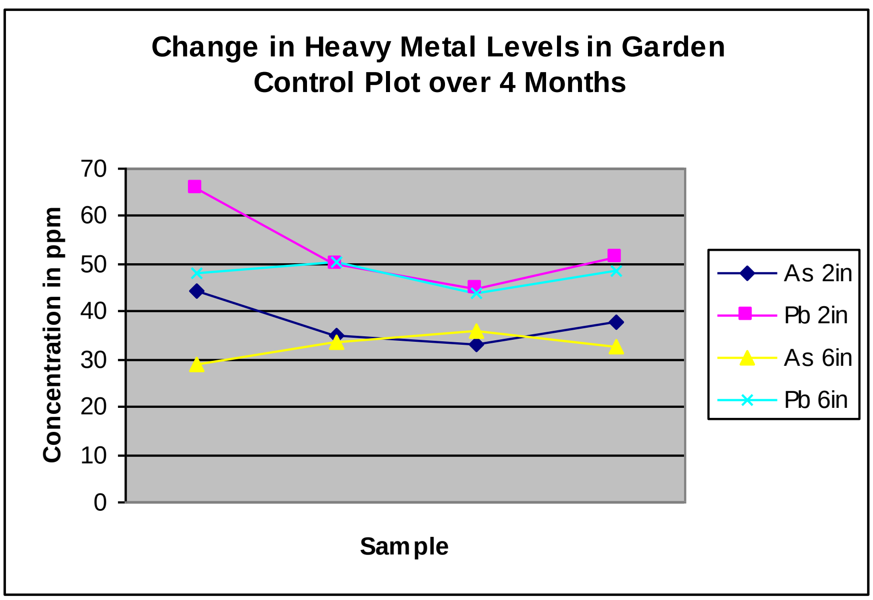


Figure 7.



Figures 5 and 6 illustrate the changes in As and Pb concentrations over the four months of mustard growth. The forest plots had a more significant change most likely due to be disturbed for the first time since the contaminated ashes fell. The range (0-200ppm) is different from the grassy area because of this large difference where the grassy area did not have as significant of a difference between the first month and the last month.

Using the Niton XRF Analyzer to test the dried plant samples, no traces of As or Pb were found. Therefore, the plants did not accumulate As or Pb in their roots, shoots, or flowers.

Summary of Results

The experiment examined whether the applications of the *B. juncea* mustards, the SCD Bioklean^{TM}, and biochar would significantly decrease the amounts of As and Pb in the soil over one growing season and whether different combinations, specifically the guild with all three treatments, would be more effective than the treatments individually. The results indicated the biochar was possibly the key component needed for the mustards to grow and the biochar also was associated with reduction of Pb in the soil. Below are interpretations of these results within the discussion section.

Discussion

Mustard Survival

It is unclear why mustards did not do well and why the ones growing in biochar survived. There are a few possible explanations for this result. Evidence of slug activity from the remaining plant stems suggested predation, but in other plots, there was no sign at all that the seedlings were ever there. Thus, it is unlikely that slugs were responsible for all the mustard mortality. This growth pattern occurred twice, with the seedlings all dying in the second round of planting in plots without biochar even when there was substantial rain. It is possible that the biochar provided the mustards with necessary nutrients for growth that were not present in the other plots

There are many possibilities as to why the mustard plants grew better in some plots than others. The plants sprouted within all of the plots, but the only ones that survived after week 4 were found in plots that also contained biochar. The week when the first planting of mustards were maturing was particularly dry. Mustards thrive in well-drained moist soils. The grassy area does not drain well whereas the previously forested area does. Biochar could have retained more water, provided better housing for the microorganisms to aid in growth, as well as provided phosphorous. Another possibility is that the biochar immobilized the As and Pb creating a non-toxic growing environment allowing those plants to grow while the others sequestered the As and Pb in a toxic form.

Often used as a soil amendment to aid plant growth, phosphorus (P) could have been added to the plots but researchers show that this addition results in the formation of insoluble complexes with heavy metals causing them to become immobile in the soil and less likely to be accumulated by the plants (Raskin et al. 1994 United States Patent). There is actually substantial research showing P as a way to immobilize Pb for many years (Yang et al., 2007). This was not desired for this research, but could be applied if immobilization is the desired affect.

Another observation showed that the plots with biochar in the forested area had a much higher plant growth rate when compared to the plots with biochar in the grassy garden area. Mustards thrive in well-drained moist soils. The grassy side garden does not drain well whereas the previously forested area does. Also, the organic layer in the forested area may be providing more of the essential nutrients for the plants. “Plants can adsorb a varying proportion of their nitrogen and phosphorous needs as soluble organic compounds. In addition, various growth-promoting compounds such as vitamins, amino acids, auxins, and gibberellins, are formed as organic matter decays (Brady and Weil p514).” These provide nutrients for plants as well as microorganisms. The amount of Nitrogen, Phosphorous, and Potassium makes or breaks phytoremediation as plants need these to grow regardless. The soils in the previously forested area may have been more optimal for growth being less disturbed and less compacted than the grassy area. The added biochar probably increased the CEC and allowing these nutrients to be more available for the plants as well.

Another issue may be lack of sun exposure in the plots. The plants may have not been getting enough light in any of the plots to grow amply. A possible alternative suggests that in some situations conducting bioremediation at a bioremediation facility, even though it costs to remove the soils and replace the soils with “clean” soils, might be more effective. The contaminated soils could be transported to a location where exposure to individuals is not as much an issue and plenty of sun is provided. Once treated, the soils could return to the site or a site with similar biology.

Discussion of Bioremediaton Treatment Results

Phytoremediation was not successful in plots without biochar. Because the plants only survived in less than half of the plots, and only in plots containing biochar, the only plots were the role of the plants could be assessed were the ones with biochar. However, plots receiving BioKlean™ need to be analyzed as well because this microbe mixture treatment also may have an effect on soil metal concentrations. For the biochar plots, there was a significant decrease ($p=0.013$, t-test) in recoverable Pb from the soil while no difference was found for the decrease in As in these plots. This decrease in Pb does not include Pb that is not recoverable by the standard method 200.8 used by Friedman and Bruye, which includes metals that are immobilized by the biochar. Therefore, from these results Pb becoming immobilized by the biochar may not be detected because it is not "recoverable" using method 200.8. The immobilization of metals by biochar occurs depending on the metals and what forms they are found in the soil. To recover the immobilized metals additional treatment is needed to solubilize the bound metals (make available for counting in the ICP-MS, or bioavailable within the environment).

The decrease in Pb in the previously forested plots is possibly a result of tilling. Even the control plots showed a significant decrease in before and after levels. The changes of metal concentration over time in the control plots (figures 5 and 6) suggests there was a reaction within the soils from tilling. The garden area had previously been tilled and disturbed so the decrease there was very minor while the decrease in the control plot in the previously forested area is greater because it has never been disturbed. Tilling can unbind metals that are immobile. Once tilled, the metals become mobile and are able to move out of the newly disturbed areas (Glass et al., 2000). Due to an observed change in the control plots in the previously forested area, this could mean that the significant values found in the previously forested area are significant due to disturbing the soils for the first time and not due to the actual addition of biochar. However, when looking only at the first sample taken from the control plots with one taken at 2 inches and one taken at 6 inches before and after treatment, change in Pb contamination was not significant within the control plot in the previously forested area or the grassy area. The changes from the initial soil samples and after treatment samples for the previously forested site were significant for Pb (p=.04, paired t-test). This supports the conclusion that the of the effect of the Biochar was significant.

The grassy area did not show any significant change for either As or Pb when examining all of the plots combined. The plots with only mustard plants did not show significant change in either area. BioKlean^{TM} + Mustard did not show a significant decrease in Pb or As when using Wilcoxin Signed Rank Sum Test or a t-test for either area.

Other natural factors could be contributing to the movement of the As and Pb. Soils can be affected by underground water movements as well as the transfer of nutrients by worms, insects, microorganisms and burrowing animals (Brady and Weil, 2008 p 451 "Soil Engineers"). Regardless, the As and Pb did show signs of becoming more mobile or immobilized, or they could also be moving within the soil ecosystem. In order to better understand what happened to the As and Pb concentrations further research should include testing of total metals in addition to recoverable metals.

Even though the soils are highly heterogeneous, there are patterns within the soil samples that show decreases and increases in the same plots suggesting different soil activity taking place at different places within the plots and between plots. This dynamic environment is also illustrated by the ANOVA results showing a high amount of variability within the same treatments. The results indicated no significant differences between treatment types. Each plot contains a different soil composition adding to the difficulty of determining if treatments acted the same way in the different locations. Due to the high variability of the initial and after treatment samples, it is difficult to answer whether the guild plots worked better or did not work any better than the double treatment plots.

Due to the small sample size and the heterogenous nature of soils, the data collected could not be verified to have a normal distribution. Therefore, the non-parametric Wilcoxin signed rank sum test was used in addition to the t-tests. Non-parametric tests are a weaker statistic. Plots containing biochar within the previously forested area show a significant decrease in Pb when using the Wilcoxon signed ranked sum test for non-normal data. The result of p= 0.034 is for the previously forested area biochar plots whereas plots in the grassy area had a p-value of 0.025 for biochar plots.

Comparing all plots containing biochar using the Wilcoxon signed rank sum test shows a significant decrease of Pb ($p = 0.004$). Plots with biochar+Bioklean™+mustard showed a significant decrease as well ($p = 0.046$). Plots with only the mustard and biochar show a significant change with reduced Pb ($p = 0.018$).

The second research hypothesis was that the guild would reduce Pb and As levels more than the other treatments. There is no evidence to support that hypothesis. Using ANOVA (single factor) to compare the means of the differences that each treatment created, there is not enough difference between treatments to show that one was more effective than the other. This also points out that the plots treated with biochar did not significantly change the Pb and As concentrations in relation to the other treatments even though the change within the plots was significant, it is not significantly different from the other treatments. Overall, these data are limited because of sample size but also because of the large natural variation in the soils.

Conclusion

This thesis provides a basis of groundwork to further the study of bioremediation for As and Pb on Vashon Island. The conclusions that can be drawn from this feasibility study are not robust, but point to possible solutions to the bioremediation of arsenic and lead. There is optimism in this research that points to the beneficial properties of biochar and the use of biochar to influence As and Pb within contaminated soils. Further testing needs to be conducted in order to know whether the Pb is becoming immobilized by the biochar. If it is immobilized, the use of biochar could be implemented in order to grow food in a safe environment where the Pb is not bioavailable. This research also illustrates what does not work in some situations for in situ remediation. Mustard plants, which are not hard to grow, need substantial amounts of sunlight as well as nutrient available soil. It is hard to say why the plants in plots with out biochar dissipated, and other plants might be better suited for Amy’s backyard ecosystem that are also good at accumulating As and Pb for harvest.

This concludes the thesis research; the next chapter provides suggestions on how to improve the approach used in this study. In addition, specific suggestions are made for future work in this topic.

CHAPTER V: Suggestions for Future Research

This chapter provides ways this thesis work could be improved in order to draw conclusions from more robust research. Even though the thesis work has been concluded, the contamination still remains. The following discussion offers a critical view of the thesis work as well as a way to expand the thesis result findings.

First of all, in order to be able to obtain more complete data, a third control plot in the design would have allowed more statistical accuracy when comparing natural soil change in the control plots to treated soils in the treated plots. It would be best to have four control plots, two in each area. Also, in order to carry out a BACI (Before After Control Impact) Paired test, multiple samples must be taken before and after treatment where as in this case the samples in the controls were taken with one prior and then 3 during the time period instead of many before and many after (Smith, 2002). BACIP provides a better way of detecting natural changes to the soils outside of the treatments and provides a stronger basis for conclusions.

Only metals recoverable by EPA method 200.8 are provided in the results. For soils, the samples must be diluted and digested. The soil digestions are done using metals grade Nitric acid, Hydrochloric acid. Ultimately, the results only reveal “leachable” metals. Leachable metals do not necessarily include all metals within the soils. It is also not completely known whether non-leachable metals are going to be bioavailable when ingested by humans.

There is a good deal of scientific research that debates whether leachable metals are equal to bioavailable metals and those that are available for plant uptake. Most research indicates that the immobile Pb is not toxic even if ingested (Magrisso, 2009; Miretzky and Fernandez-Cirelli, 2007, Yang et al., 2007). Still more research needs to be done in order to understand what forms of immobile Pb and As are non-toxic to humans. Also, it is important to note that the immeasurable, immobile Pb may become mobile over time and it is very difficult to know how long the Pb will remain bound within the soils. Even with the Pb bound the contamination is still there, and through time could become bioavailable. Future research should involve total metals analysis instead of recoverable metals. This can be done with an additional acid leaching process using hydroflouric acid. Also, it would be best to have tested the plants for any Pb or As concentrations found within the roots, shoots, leaves and florets to compute a mass balance for each metal.

How this Experiment Could Be Improved

Enhance irrigation system to be set on a timer. In order for the timers to work barrels need to be raised higher to create more pressure allowing the timers to work properly.

Find a better plant species. Another mustard species would work better and it would be best to get more than 200 seeds of the varieties known to accumulate As and Pb. After seeing the growth of the mustards *Brassica juncea* it was noted that they did not grow very large in the leaves or tall in the stems as was expected. It was later discovered in the book Trace Elements In Soils edited by Peter Hooda, that *B. juncea* is actually an over wintering crop. It is to be planted in late fall and flowers in the spring (p330). Thus, a better plant more suited for growth in the Pacific Northwest is desirable for phytoextraction.

Broadcast seeds rather than plant seeds in rows.

Do a more in depth search of how to obtain more than 200 seeds of cultivar 426308. Either ore seeds need to be required or 200 seeds need to be planted and grown to generate enough seeds to conduct the research.

Look at companion planting with natural chelating grasses. Some grasses can aid in the mobilization of metals while others actually sequester As and Pb. More research needs to be done in this area for Vashon to secure high use areas such as parks.

Use a more specific blend of microorganisms created for heavy metal remediation of As and Pb. Some research suggests that using a blend of microorganisms containing *Pediococcus dextrinicus* and *Pediococcus acidilactici* would be more effective due to these micoroorganisms ability to change As and Pb bonds within various mediums.

Make a more personalized blend of microorganisms for the plant being used. None of the microorganisms in the microbial inoculant are actually found in the rhizosphere of Brassica juncea. (Belimov et al., 2005)

Inoculate biochar with microorganisms before adding as soil amendment. Further research on alternate ways to apply microbes with biochar will likely improve microoganism affect in adding plant growth.

Use different laboratory soil tests to determine total metals instead of only recoverable metals allowing better determination of adsorption effects/ immobilization of Pb by biochar. Total metals analysis can be preformed using EPA method 200.8. Total metal analysis is rarely performed because it requires tedious and extremely hazardous sample preparation to completely dissolve all soil minerals and has increased monetary costs. Two accepted procedures are heating the sample with a mixture of HF and HNO3 or fusion (high temperature heating) with lithium metaborate. Both produce fairly toxic solutions containing the dissolved metals. Total metals analysis will help answer questions related to how the biochar is affecting the Pb within the soils.

Additional testing: Test soils for pH more widely using a pH probe. In order to best understand how biochar is reacting within the soils more tests need to be done to understand soil composition. Metal speciation tests are ideal but very costly. Weigh soils before and after if possible and test for soil porosity before and after application of biochar.

Overall larger sample sizes needed.

Additional Experiments

Biochar and Mustards

Results indicated that Pb was significantly affected by the soil amended with biochar and microorganisms in combination with the biochar. Thus, a feasibility study focused primarily on the affects of biochar is needed. In order to provide a more in depth understanding, a more rigorous sampling procedure will be used and the test area will be set up in one or two large plots with embedded subplots treated with slightly different amounts of the biochar. The plots could consist of 12 meters of contaminated soils, tilled. Then it will be subdivided into control areas of 1 square meter with no biochar added interdispersed with plot areas with the biochar added as it was in this prior feasibility study. This would allow better comparison of the soils that were only tilled and those with the biochar and mustards grown in them illuminating the affects of only tilling compared with the affects of tilling and adding biochar. As an alternative, the plots study area could be set up with one side of the 12 square meters being the control and the other side being treated with the biochar. Then the sampling would be done with random sampling from each side with 6 samples from each made up of composite samples (i.e.: taking 3 samples and mixing them together to form one composite homogeneous sample and repeating this 6 times for each side).

Prior to tilling or adding biochar rigorous sampling should be done with an XRF Niton Analyzer to accomplish a large sample size as the base range of contamination. This can be done by removing the topsoil every 6 inches in a grid pattern to create a base map. Then, there should be a round of three samples taken after tilling and then samples taken in control plots and soil amended plots right after the addition and after plant harvest. For this research, it is vital that the CEC and AEC be tested before and after treatment. This can be done by air drying s grams of the soil samples and sending them to a lab with an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) machine in order to count the exchangeable cations and anions.

It may be that the biochar is merely adding biomass to the plots thus reducing the overall concentrations of arsenic and lead. However, if this were the case the arsenic concentration would decrease significantly as well. By conducting a more in depth biochar analysis, these questions will be more clearly answered.

One issue that should be noted when leaving large tracts of contaminated soils open to the air is the possibility of wind taking the contamination into unwanted areas as well as increasing the likelihood of personal contamination through inhalation by researchers. Also, in order to harvest and test after the tilling has occurred there will be definite contamination of boots and clothing used in the field. This is a serious issue and illustrates why separate plots were used in the initial feasibility study. Perhaps plastic bags can be worn around the boots and then the contaminated soils can then be washed off back into the soils after use.

Testing the Effects of Tilling

It would also be interesting and illuminating to conduct research aimed at the affects of tilling and sampling to better understand how As and Pb varies in concentration within the soil. This experiment would also include rigorous sampling. The area being remediated would first be cleared of just the sod layer. Then an XRF Niton Analyzer would be used to map out the surface soil contamination taking readings at every 6 inches across the area. The area would then be subdivided into smaller sampling plots each 1 m squared. From these plots soil samples will be taken in the same fashion as in the feasibility study with 3 samples mixed together at 2 inches and three mixed together at 6 inches to create two samples from each plot for the initial samples. Then the area would be tilled. Every 3 weeks samples will be taken over a one year period. Once at the end of each season, the area will be mapped by the Niton XRF Analyzer. The initial map will be created at the end of Winter and the final map will be created at the end of Fall while two maps will be done in between, one at the end of Spring and one at the end of Summer.

Conduct *ex situ experiments*

Create a small laboratory style test by digging soil up and putting it in a deep plastic tub on site. Apply the same amendments and soil testing protocol as *in situ*. This will allow a better idea of how well the amendments are working at the site without the uncontrolled movement of soils by “soil engineers”. This can help researchers form a better projection of the length of time needed and how much of the soil is being affected by soil movement in and out of the area.

Selection of alternative Species

Note on *Pteris vittata*

The ladder fern *Pteris vittata* was the original choice as the phytoremediation hyperaccumulator for this research. However, upon further investigation not only was the plant rather expensive at $5 (with little time to invest in growing and pollinating to grow our own), the plant was a potential invasive species for the Pacific Northwest region. It was listed on the Florida Invasive plant list by the Florida Exotic Pest Plant Council in 1999 and remains there today (FEPPC, 2009). Also, there is little evidence that it would accumulate Pb as well.

Through a conversation with Norm Peck, a state employee with the Washington State Department of Ecology, information was found on a 2-year study done on Vashon Island using the ferns and found they did not accumulate Pb . They are hard to grow in this climate because they need full sun for ½-2/3rds of the day and do not last well through the winter causing less As to be accumulated the following year (Department of Ecology website, 2005). He also expressed concerns over the ferns being a hazardous waste after harvest. They need water during the growing season and Washington's sunny growing season is usually dry.

A common concern is what to do with the plants after they have been harvested. The argument is often answered by the idea that plants are lighter to transport than soil. Also, there is a numerous amount of research on extracting the contaminants from the plants for use in laboratories/and other chemical uses. One issue the fern research illustrates is the difficulty of determining whether or not the soil is being remediated due to soil variability. "The young ferns planted in April through June this year contained less than 1 part per million (ppm) As in their fronds at the time of planting. At season's end, the As concentration in the fronds ranged from 828 to 16,000 ppm (dry weight) (Chinese Brake Ferns, 2005). Even though the plants accumulated mass amounts of As the test plots did not show a corresponding decrease.

> In some plots, arsenic concentrations appear to have increased. However, this is likely due to the high variability of arsenic in soil as shown in our past Tacoma Smelter Plume studies. This variability makes determining soil removal rates difficult, especially over only one year and two sample sets (baseline and harvest in 2005). Mass balance calculations indicate a slight reduction, though less than predicted. We cannot point to a definable reduction in soil arsenic concentration in the test plots this year. (Chinese Brake Ferns, 2005).

Grass

Using grass as a phytoaccumulator provides both phytostabilization and hyperaccuualtion. The grass *Agrostis tenuis* is known for its ability to be tolerant in Cu waste contaminated soils (Salt et al., 1995). *Agrostis castellanai*, Colonial bentgrass has been shown to accumulate As and Pb (LID Technical Guidance Manual). These grasses may have less biomass and take a longer period to remediate, but allows for easy harvest through mowing and collection in a lawn mower bag.

Mycoremediation

A reassessment of the site for mycoremediation would be beneficial. Mycorrhizal fungi have been coevolving with over 95% of the plants on earth through a symbiotic association, exchanging important nutrients. Mustard plants happen to be in the 5% of plants that do not form relationships with fungus (mycorrhizae.com). Thus, no fruiting mushrooms or mycorrhizael mushrooms were used for this part of the study. Mentioned before, mycoremediation was originally a part of the design for the feasibility study and will possibly be applied after the second round of mustards are harvested depending on whether the mustards accumulate or not. If the mustards hyperaccumulate As and Pb mushrooms might hinder this process. However, if they do not hyperaccumulate arsenic or lead mushrooms in combination with another plant such as a native fern, might make a better guild.

Fungus has been shown to actively accumulate heavy metals out of soils. “Fungi in particular may be able to accumulate…metal(loid)s into their cells. The intracellular uptake of metal ions from a substrate into living cells, known as bioaccumulation, may lead to the biological removal of metals by fungi (Adeyemi, 2009)”. Adeyemi demonstrated the importance of fungus in the detoxification of arsenic by rendering the arsenic in the soil from a soluble to an insoluble form. Two mushrooms are known to hyperaccumulate As: Shaggy Mane and Shaggy Parisol (*Macrolepiota rhacodes)*. Shaggy parisol also accumulates Pb (Stamets, 2005). Both of these mushrooms have fruiting bodies which occupy the very top layers of the soils. Thus, it would be difficult for them to access deeper toxins within the soil. This issue might be addressed by a good plant/mushroom combination where the plant will bring the metals toward the surface with its root systems. Shaggy parasol is native to the Puget Sound area and is commonly found in polluted soils, hard ground, grassy areas, and trails. This mushroom is able to grow in a range of temperatures in the environment and grows from late fall to early winter (Stamets, 2005).

A great deal of research has been done on the relationships between mycorrhizae and biochar where the char has been used with great success as a carrier substrate for arbuscular mycorrhizal fungi (Ogawa, 1994). The mycorrhiza obtain carbohydrates from the plant and in exchange help the plant get access to more nutrients from the extensive coverage of mycelium in the soil. Mycorrhiza can also transform phosphate to plant available forms (Brundrett, 2003) and help reduce infection by pathogenic fungi (Matsubara et al., 2002). Mycorrhizae abundance increases with biochar additions (Warnock, 2000). This increase could be from reduced competition from saprophytes or protection from grazers in micropores (Saito, 1989). However, using microorganisms with the mycorrhizae may create unwanted complications between the two resulting in competition for survival. A study using only biochar and mycorrhizae may have interesting and significant results.

Afterward: Knowledge and Application

Eden reframed is a community ecoart project located on Vashon Island that creates awareness about remediation and permaculture. Working with Beverly Naidus, the Home Remedy Project gained attention through work done at the Eden Reframed site. There, we created deep plant beds that allowed for water retention. We planted one side of the garden with edible local plants and the opposite side with plants that were known local remediators. Signs at the site provide information about heavy metal contamination as well as information on other plants that remediate for fecal coliform or high methane, even for depression. Working on Eden Reframed during the Home Remedy Project allowed a more free expression of the heavy metal issue that brought people to a better understanding of the issue in a safe and open environment.

The heavy metal contamination is only a small part of the overall destruction that has occurred through industrialization. Being able to grow food locally in healthy soil is vital and being threatened globally. Luckily, bioremediation is showing more positive results when dealing with pesticides and petroleum pollution (Geetha and Fulekar, 2008; Gavrilescu 2005; Seech et al. 2008) and there must is still be a way to remediate for As and Pb using sustainable solutions.

There is a large gap between bioremediation research, the knowledge it has generated and the application of this knowledge to the clean up of soils in the real world. Billions of dollars have been provided to aid in the “cleanup” of As and Pb but still has not provided for every area contaminated especially privately owned land. “’Today’s landmark enforcement settlement will provide almost one billion dollars to clean up polluted Superfund sites," said Cynthia Giles, Assistant Administrator for the EPA’s Office of Enforcement and Compliance Assurance (December of 2009). "This will mean cleaner land, water and air for communities across the country.’(EPA Compliance)” It is distressing to see that after 30 years of the ASARCO smelting plant being closed the people of Vashon and Maury Island are still being left to deal with the contamination on their own without seeing many of the benefits from this funding.

Even though there are established positive results for phytoremediation on metal extraction, it does not appear that this knowledge is being applied. One reason for this is the difference between in situ remediation and research done in a controlled, lab/greenhouse environment. Currently, the remediation work being done by The Washington Department of Ecology involves digging and transporting the contaminated soils to landfills which does not truly "clean" the contamination, but just moves it to another location.

Going deeper in order to find out why bioremediation of heavy metals was not occurring, Illya Raskin was called because he owns a patent on specific *B. juncea* species that are proven to remediate for As and Pb Never hearing back from him as to why the remediation with *B. juncea* was not at all common a paragraph was finally found in the book *Trace Elements in Soils*, the 2011 edition edited by Hooda. The patent was licensed to Phytotech, Inc. who conducted the field research. Results concluded that the plants were able to hyperaccumulate Pb at a high rate in nutrient hydrolic water, but not in soils. In response to this, chelating agents were added to the fields, which induced the extraction of the Pb by the *Brassica juncea*. However, further research showed that the result of adding chelating agents caused Pb to leach into groundwater in high concentrations.

Hooda et al. explains, "It is very unfortunate that many researchers were misled by these studies to believe that Pb Phytoextraction might be practical. Literally hundreds of papers have since been published on Pb Phytoextraction with different plant species and different chelating agents. None have demonstrated a cost-effective and environmentally acceptable Phytoextraction technology for soil Pb. (p330)"

With that said, biochar still could be a possible solution to the issue of Pb leaching into groundwater during the remediation process and further research should go into finding the right combinations in order to bioremediate Pb safely or contain it safely on site in order to grow food in a safe environment. With the 30 years that the ASARCO smelting plant has been closed, if bioremediation techniques had been implemented right away using the right grass and the *Pteris vittata* ferns, it is possible the plants would have taken much of the contamination out of the soils. The need for a process that is fast and monetarily beneficial to involved parties has left Puget Sound with a very expensive mess. Biormediation is a way to remediate this issue for good, but most methods are very slow and require decades to be completed. This study highlights the difficulties of conducting bioremediation for As and Pb on Vashon Island and suggests the importance of more research in this area and ways it can be accomplished.

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APPENDICES

Additional Information

USDA National Germplasm System

The mission of the National Plant Germplasm System (part of the Germplasm Resource Information Network GRIN) of the USDA is to "serve as the information system for the documentation of plant, insect, animal and microbial germplasm maintained by the U.S. National Genetic Resources program". Plant germplasm is collected from around the world. Then curators and scientists preserve, evaluate, and catalog this germplasm and distribute it to people with a valid use (Seeds for Our Future 1996). "The National Plant Germplasm System is devoted to the free and unrestricted exchange of germplasm with all nations and permits access to U.S. collections by any person with a valid use (Seeds for Our Future 1996)" such as plant researchers and breeders. However, there are some limitations. In order to maintain the collections they only distribute 200 seeds of any particular variety to each individual request. When the seeds were requested for this research only 200 of the 426308 *B. juncea* seeds could be obtained. At least 1200 seeds are needed for this study. Although the cultivar 426308 found by Kumar et al. was the best known accumulator, another variety from a source able to provide enough seeds was chosen. If the study was postponed a year the NPGS seeds could be used as germplasm to provide the seeds for the research one year later.

Grant Proposals

SEEDS
Social Ecology Education & Demonstration School
PO Box 2895
VashonIsland, WA 98070
206·949·4786
www.socialecologyvashon.org

April 30, 2010

The Harris and Frances Block Foundation
491 Ennis Hill Road
Marshfield, VT 05658

Dear Harris and Frances Block Foundation Board,

I am impressed by the breadth of efforts to which you have lent support, encompassing such areas as forest preservation, food security, anti-nuclear and anti-genetic engineering, education on peace and justice and on militarism, community composting, revitalization of traditional ecological cultures, outreach and education for at-risk teenagers, and independent media, and more. Your foundation is contributing greatly to overcoming a wide range of social and environmental problems by supporting the work of these vital grassroots efforts—for which funding is unfortunately not easy to come by in today's world, and is therefore absolutely crucial. The values of environmental stewardship, social justice and responsibility you express through this kind of support are very much in alignment with the values we hold at SEEDS, and I greatly appreciate the opportunity to collaborate with you.

Bob Spivey

Founder and Board President
Social Ecology Education and Demonstration School (SEEDS)

Harris and Frances Block Foundation

Grant Application

Cover Sheet

1. Organization name: **Social Ecology Education & Demonstration School (SEEDS)**
   Date: **April 28, 2010**

2. Address: __PO Box 13126________________

   _Burton, WA 98013______________________

3. Contact Person & Title: Bob Spivey, President

   Phone #___206-949-4786_____________

   Email ___bobespivey@gmail.com_____

4. Person responsible for the program:__Jenn Coe_________

   Phone # ___206-384-0973____________

   Email ___Jenntree@gmail.com_______

5. Project Title: ____SEEDS Bioremediation Home Remedy Program_____

6. Estimated project start date: __June 2010_______________

7. Amount requested: _______$6,535_____________

8. Project summary:

The SEEDS Bioremediation Home Remedy Program aims to demonstrate the effectiveness of low technology phyto- and myco-remediation techniques in soil contaminated by arsenic and lead on Vashon Island to levels below the stringent standards set by Washington State. The program will demonstrate an accessible, replicable model for remediating a soil condition of great concern not only to those living on Vashon, but to many areas throughout the Puget Sound. The research plots will serve as a hands-on practicum site for education on remediation approaches, as well as permaculture and other approaches that embody grassroots, community-controlled approaches to sustainability. The project for which we are currently seeking funds is the first phase of a project that will provide ongoing training in remediation and restoration throughout the area with particular focus on low-income communities.

SEEDS Bioremediation Home Remedy Program

History and Mission of the Social Ecology Education & Demonstration School

Headquartered on Vashon Island in Washington State, the Social Ecology Education and Demonstration School (SEEDS) helps meet the urgent need for an educational ecological project aimed at both local and global communities. The mission of SEEDS is to develop and offer educational experiences that enhance people's abilities to knowledgeably and creatively address the interwoven social and ecological crisis of our time. Through an intensive and interdisciplinary study, participants gain a critical and comprehensive understanding of social and ecological reconstruction. SEEDS provides participants with opportunities to test various reconstructive strategies by means of individually designed practicum learning experiences.

Beginning last year, SEEDS has played a key role in the development of what has come to be known as the "Vision for Vashon." A comprehensive community development effort, Vision for Vashon sparked citizen work groups in affordable housing, community health, community solar energy, alternative currency systems, food security, and grassroots sustainability projects. The food security/food sovereignty group has become a particularly active and robust group, and SEEDS' goals in relation to food sovereignty include the promotion of collaborative farming/gardening, and education in permaculture approaches.

Problem: Heavy Metals and Bioremediation

Soil remediation is a necessity for the Vashon community in order to enable reliable, healthy and local food sourcing, an imperative especially for our Island community. One particular challenge we face is the widespread contamination of soils with heavy metals from the ASARCO smelting plant, operated from 1887 to 1986 in Ruston, Tacoma, Washington (Glass, 2003), across the water from Vashon Island. The southern half of the island was under the smelter plume windfall area, leaving it contaminated with mass amounts of the heavy metals arsenic and lead. The EPA has designated the ASARCO site a Superfund site since 1979 and has documented the contamination of lead and arsenic in the adjacent areas. The Washington State Department of Ecology (WSDOE) found concentrations of 360 parts per million (ppm) of arsenic and 1300 ppm of lead on certain soils on the south side of Vashon Island. This level of contamination is well over the EPA safe limit for bare soils and is more than three times the limit for areas with children. It is far above Washington State's limit of 20 ppm concentration standard for arsenic and the state's 250 ppm for lead (WSDOE).

SEEDS has designed a research-based educational project to discern and implement a system for removing these contaminants using bioremediation methods. Our priority is that the system be affordable, effective and replicable to other communities. We will freely share our findings with other areas facing the challenge of contaminated soils, and we anticipate that our research will benefit countless communities in their quest of restoring land for food production and other community needs.

Bioremediation Home Remedy Program: Goals and Objectives

SEEDS envisions a permaculture "make-over" of bioremediated garden spaces that have been largely abandoned through concerns about contamination. The Home Remedy Program aims to provide a proven, replicable, and affordable model for remediating soils contaminated with the arsenic and lead. Bioremediation techniques are inexpensive and holistic, providing healthy living systems for daily life and food production.

Our goal for the Home Remedy Program is to demonstrate that contaminated soils within the smelter plume area of Vashon can be remediated through ecological and accessible low-technology means for safe food production, according to the rigorous standards of Washington State, which are considerably more stringent than the EPA standards. The objective of this particular study is to compare effectiveness between low and high technological techniques using three different ecological methods: funguses, micro-organisms and ferns. We will then further study these methods' effectiveness when working together, to view their effectiveness in a "guild." A guild is a permaculture term meaning "harmonious assembly of species (Mollison, 60)".

These test sites will be transformed into sites for on-going community education in both bioremediation and permaculture approaches, providing a positive and lasting impact not only on the health of Vashon Island's 13,000 residents but on other communities facing similar challenges. The program will also promote food sovereignty, which means not merely food security (which might be obtained through food transported long distances), but the maximizing and democratizing of local resources for food production.

Study Design

The Bioremediation Home Remedy initial study will compare bioremediation techniques using low technology (locally based and sustainable resources) with those using higher technology techniques (imported and more expensive resources) within contaminated soils on Vashon Island. By testing three techniques at a low and high technological level, a better understanding will be gained at the local level of different cost variables as well as the bare minimum needed to successfully bio-accumulate the contaminants. The purpose of the two technological levels is to provide replicable practices for people with varying funding resources, while maintaining scientific accuracy. We hypothesize that there will be negligible differences between the low and high technology methods of bioremediation. We also hypothesize that the guild bioremediation plots will be more effective at remediating the soil than the individual specie and species plots.

Methodology

SEEDS researchers will start with 18 one meter square plots, inoculated with three different sets of organisms including one control plot. There will be two plots inoculated with the fungal spawn of Shaggy mane (*Coprinus comatus*), two plots inoculated with specific blends of microorganisms, and four plots planted with ferns. We plan on comparing the local Lady fern (*Athyrium filix-femina)* with the non-native Chinese brake fern *(Pteris vittata*) which has been studied for successful arsenic remediation. There will be two plots with Lady ferns with Shaggy mane mushrooms, two plots with Lady ferns and microorganisms, and two plots with microorganisms with Shaggy mane mushrooms. The last plot for each set will be a guild of all three organisms. Low technology methods and plots will consist of hand tilled plots, wild harvested Shaggy mane mycelium to be cultivated, compost tea from local sources and wild harvested Lady ferns. The high technology plots will be comprised of purchased Shaggy mane spawn, a specialty blend of BioKleen probiotics, and purchased Lady ferns. For the high technological plots a mechanized tiller will be used.

The study will be conducted over an initial six month period to allow for a full growing season to be completed. Also, as a part of the research process, a six month education program happen, beginning in June and culminating with a public workshop that highlights the bioremediation program within the framework of "Radical Sustainability," a vision SEEDS has adopted from Scott Kellogg and Stacy Pettigrew of the Rhizome Collective. Radical Sustainability champions the necessity that resources required for meeting people's needs be designed, built, maintained, and controlled by those who use them, and materials be likewise accessible, low-cost, and shared. The SEEDS Home Remedy Program works toward this vision. The Home Remedy Program will also feature an educational art exhibit dramatizing the processes and benefits of bioremediation, designed by noted eco-artist Beverly Naidus.

Analysis

Testing will be done with a Thermo Scientific Niton® XRF analyzer to conduct Positive Material Identification (PMI), with five percent of the samples sent to a King County lab for verification, as dictated by the EPA. The analysis procedure will consist of the following steps:

- We will take nine samples from each plot for initial testing. Each sample will be sieved and grinded down with mortar and pestle according to the EPA's procedural guidelines. Then the nine processed samples from the same plot will be mixed together and sub-sampled three times, repeating this for each plot's group. The sub-samples will be compacted into a small testing container and analyzed three times for an average. We will continue this soil analysis every month for six months.
- Shaggy mane mushrooms will be harvested after 3-4 months upon their fruiting. Each mushroom from its specific site will be dehydrated and powdered, then mixed with samples from its plot and sub-sampled three times. Each sub-sample will be compacted into a small testing container and analyzed three times for an average, with five percent of the samples sent to a King County lab. The same procedure will be used for the Lady ferns from each plot.
- The categorical data sets will be analyzed with a Chi-squared analysis to insure statistical significance. Our categorical independent variables are each plot, while our dependent variables will be the fruiting mushroom bodies, aerial portions of the ferns and the control plot. The before and after levels of lead and arsenic in each plot will then be compared to the control plots using T-tests which is typically used to compare the before and after samples. In order to observe the changes that occur over time, measurements taken each month will be plotted on an x-y scatter plot to show changes in concentration.
- We will compare the differences between low and high technology methods and each method's ability to remediate the soil. The guild plots with all three sets of organisms will be compared. Each plot will be examined by progression of

change over time and how it effected the remediation. Using a Pearson's test we will analyze the correlation between the fruiting mushroom bodies and the soil in which they grew. The same test will be done for the aerial portion of the Lady fern and its soil. We will also conduct Analysis of Variance (ANOVA) which compares three or more means in order to find the most efficient process.

Evaluation

The Bioremediation Home Remedy Program will be evaluated on three main principles. The first criteria for evaluation will be the ability of the bioremediation process to successfully clean the contaminated soils, confirmed by monthly testing of the soil and aerial bodies of the fungi and Lady Ferns. The second component will be the collection of scientific data to gain a better understanding of bioremediation processes and interactions between species. This will give residents, as well as local, state and federal agencies better tools for remediating contaminated soils. The last important principle to be evaluated will be the level of community involvement and education. This will be assessed by the attendance at three public workshops held by SEEDS at the bioremediation sites and by the community's feedback and education about the study, including articles in the local newspaper, outreach at the local farmer's market and other venues, and updates on the SEEDS' website. Further evaluation will be done as the Home Remedy Program is put into practice throughout the community.

Sustainability

The Bioremediation Home Remedy Program uses and addresses the three pillars of sustainability: incorporating the importance of community education and empowerment, providing economic support and resources, and keeping the ecological environment a priority. SEEDS is funded by workshop tuition fees and a steady stream of small and large private donations, organized partly through membership in our Rhizome Club. SEEDS has also received several small grants and developed earned income products and services. Volunteers and a pulsing Puget Sound community provide the momentum for SEEDS to continue to promote educational experiences that make lasting changes to better the global community.

SEEDS Bioremediation Home Remedy Program Budget			
	High Tech	Low Tech	Total
On-site work			
Mechanized tiller	$390.00		$390.00
Shovels (hand tilling)		$300.00	$300.00
Site Development (preparation)	$75.00	Donated	$75.00
Labor	Volunteers	Volunteers	
Remediation organisms			
Fungi Culture	$250.00		$250.00
BioKlean Culture	$2,100.00*		$2,100.00
Chinese Brake Fern	$155.00	$155.00	$310.00
Lady Fern harvest and propagation	$275.00	$275.00	$550.00
Homegrown Culture		$30.00	$30.00
Compost Tea		$80.00	$80.00
Other associated costs			
Transportation	$250.00	$250.00	$500.00
Lab testing	$300.00	$300.00	$600.00
Education	$350.00	Donated	$350.00
Unforeseen Expenses	$250.00		$250.00
Total	$4,395.00	$1,390.00	$5,785.00

***Estimate from a supplier of probiotic microorganism blends, based on suggested dosage and delivery costs.**

INTERNAL REVENUE SERVICE
P. O. BOX 2508
CINCINNATI, OH 45201

DEPARTMENT OF THE TREASURY

Date: OCT 11 2007

THE SOCIAL ECOLOGY EDUCATION AND
DEMONSTRATION SCHOOL
C/O BOB SPIVEY
P.O. BOX 13126
BURTON, WA 98103

Employer Identification Number:
20-4740965
DLN:
607197052
Contact Person:
JACK D NEITZEL ID# 95127
Contact Telephone Number:
(877) 829-5500

Accounting Period Ending:
December 31
Public Charity Status:
170(b)(1)(A)(ii)
Form 990 Required:
Yes
Effective Date of Exemption:
April 23, 2006
Contribution Deductibility:
Yes
Addendum Applies:
No

Dear Applicant:

We are pleased to inform you that upon review of your application for tax exempt status we have determined that you are exempt from Federal income tax under section 501(c)(3) of the Internal Revenue Code. Contributions to you are deductible under section 170 of the Code. You are also qualified to receive tax deductible bequests, devises, transfers or gifts under section 2055, 2106 or 2522 of the Code. Because this letter could help resolve any questions regarding your exempt status, you should keep it in your permanent records.

Organizations exempt under section 501(c)(3) of the Code are further classified as either public charities or private foundations. We determined that you are a public charity under the Code section(s) listed in the heading of this letter.

Please see enclosed Publication 4221-PC, Compliance Guide for 501(c)(3) Public Charities, for some helpful information about your responsibilities as an exempt organization.

Revenue Procedure 75-50, published in Cumulative Bulletin 1975-2 on page 578, sets forth guidelines and record keeping requirements for determining whether private schools have racially nondiscriminatory policies as to students. You must comply with this revenue procedure to maintain your tax-exempt status.

If you distribute funds to other organizations, your records must show whether they are exempt under section 501(c)(3). In cases where the recipient organization is not exempt under section 501(c)(3), you must have evidence the funds will be used for section 501(c)(3) purposes.

Letter 947 (DO/CG)

If you distribute funds to individuals, you should keep case histories showing the recipient's name and address; the purpose of the award; the manner of selection; and the relationship of the recipient to any of your officers, directors, trustees, members, or major contributors.

Sincerely,

References Cited

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SEEDS: http://www.socialecologyvashon.org/index.php

Washington State Department of Ecology (2002). **Washington State Department of Ecology Toxics Cleanup Program Tacoma Smelter Plume Site, King County Mainland Soil Study Executive Summary, March 2002.** http://www.kingcounty.gov/healthservices/health/ehs/toxic/TacomaSmelterPlume/background.aspx#timeline

SEEDS
Social Ecology Education
& Demonstration School
PO Box 2895
VashonIsland, WA 98070
206·949·4786
www.socialecologyvashon.org

May 13, 2011

The Harris and Frances Block Foundation
491 Ennis Hill Road
Marshfield, VT 05658

Dear Harris and Frances Block Foundation Board,

Bob Spivey

Founder and Board President
Social Ecology Education and Demonstration School (SEEDS)

The SEEDS Bioremediation Home Remedy Program aims to demonstrate the effectiveness of low technology phyto- and myco-remediation techniques in soil contaminated by arsenic and lead on Vashon Island to levels below the stringent standards set by Washington State. The program will demonstrate an accessible, replicable model for remediating a soil condition of great concern not only to those living on Vashon, but to many areas throughout the Puget Sound. The research plots will serve as a hands-on practicum site for education on remediation approaches, as well as permaculture and other approaches that embody grassroots, community-controlled approaches to sustainability. The project will now be entering into the second year of the first phase where it aims to provide ongoing training in remediation and restoration throughout the area with particular focus on low-income communities.

Bioremediation Home Remedy Program Update

SEEDS Bioremediation Home Remedy Program Update

History and Mission of the Social Ecology Education & Demonstration School

Headquartered on Vashon Island in Washington State, the Social Ecology Education and Demonstration School (SEEDS) helps meet the urgent need for an educational ecological project aimed at both local and global communities. The mission of SEEDS is to develop and offer educational experiences that enhance people's abilities to knowledgeably and creatively address the interwoven social and ecological crisis of our time. Through an intensive and interdisciplinary study, participants gain a critical and comprehensive understanding of social and ecological reconstruction. SEEDS provides participants with opportunities to test various reconstructive strategies by means of individually designed practicum learning experiences.

SEEDS has played a key role in the development of what has come to be known as the "Vision for Vashon." A comprehensive community development effort, Vision for Vashon sparked citizen work groups in affordable housing, community health, community solar energy, alternative currency systems, food security, and grassroots sustainability projects. The food security/food sovereignty group has become a particularly active and robust group, and SEEDS' goals in relation to food sovereignty include the promotion of collaborative farming/gardening, and education in permaculture approaches.

Over the last year, the Home Remedy Project has promoted the development of a working understanding of how to create healthy soils on Vashon allowing for the progression of research in the area of bioremediation within the community. By bringing experienced remediation specialists, such as Howard Sprouse from The Remediators to consult on the project as well as supporting interested researchers in the improvement of the initial Bioremediation Home Remedy design.

Over the winter we have researched, compiled resources, contacts, consultants and revamped our methods and procedures for this project to get better results and provide a more effective model for our community.

Lessons Learned and the Initial Impact of the Project

The Home Remedy Program is still a blend of recent bioremediation science, permaculture design principles and community driven needs. Over the past year our research has furthered our understanding of what will work best for our communities needs while keeping in line with scientific principles. This approach has taking time to properly plan and implement. We have met only a small portion of the goals we set out to meet in our proposal last year but intend to meet our goals this year. Implementing such an elaborate experiment has proven more complicated than first considered. As more research was conducted over the months of May and June of 2010, discoveries were made such as the invasiveness of the ferns *Pteris vittata*, and how to properly inoculate mushrooms outdoors. Due to timing and the need to conduct more in depth research, as well as acquire all of the necessary equipment for the study, the desired timeline for starting the main body of research has been delayed to this year in order to work with the growing season.

Last fall 7 plots were started at our site on south Vashon Island as a short term experiment to see the affects of homemade compost teas and professional grade teas. The time frame of the experiment was only 8 weeks and did not produce any qualitative data

that is of any statistical significance. The short time frame was due to a late start in early October and the end of our summer /fall season in late November. This did, however, give us insight into improving our sampling and inoculation methods. We experienced some issues with using the XRF Analyzer due to its unavailability. We also had a very cold and wet fall that did not bode well for our mustard greens.
With the return of the Niton XRF Analyzer to the Seattle area, we have been able to locate the most contaminated locations for plot placement and, one year later, the project is moving forward with sprouts in the ground and the baseline soil samples being analyzed.

Accomplishments made within the program include a better understanding of the complicated multi-specie guilds. An improved set of guild combinations have been formulated that are more likely to perform and be ecosystem safe while still being affordable. We have turned away from the more complicated low and high technology comparisons toward a method that is low and high technologies combined in a medium cost. Now we are using our plot space to assess the guild combinations rather than both the factors of the guilds and the technology methods at the same time. This will give us more emphases on the ability to remediate rather than the affordability. Also, all of the necessary equipment has been purchased and prepared for research implementation.

More people are now aware of the research and are becoming involved. Professors form the Evergreen State College are excited for the research and are providing support through education and access to lab facilities through their Masters of Environmental Studies student, Shannon Clay, who is writing her thesis on this bioremediation project. Freidman and Bruye, a local Seattle Lab who are EPA approved for soil testing have offered to do part of the soil testing pro bono to ensure readable results of the study. Three presentations have been given on the issues around the ASARCO plume pollution and the Bioremediation Home Remedy Programs efforts to remediate these soils and to further educate the communities affected. These have highlighted the questions that still remain within this newly developing field and provided community connections in support of the work. Home Remedy has also been highlighted in the SEEDS Spiral Visions Permaculture Soils Workshop weekend and in Beverly Naidus's eco-art edible forest garden remediation instillation at a local skate park. Undergraduate students are becoming involved in the process from both The Evergreen State College and Seattle Central to help with biochar inoculation, soil and plant testing, and the implementation of the project at the site.

The biggest lesson being learned through the Bioremediation Home Remedy Program is that of patience as well as thoroughness of study design. Phytoremediation has most often worked over long periods of time with the most successful remediation being done after three years of the hyperaccumulators growing in the contaminated spots. Our project aims to have a more efficient process by creating healthier soils for the plants to grow in even in these stressed conditions. We must acknowledge that this is a new field of study and most research has been conducted in labs rather than on site. We hope to see strong results within the first year, but can not guarantee that will be the case. As in all science, we are working within a hypothesis that can be limited in our human understanding of the biological processes that take place within soils and root communities. Also, it may take more time to actually but ideas into action especially when working with different people over long distances. It has been helpful for SEEDS to keep making timelines and schedules to meet deadlines, but to recognize when it is okay to allow plans to address the schedules life presents us with.

Sustainability

The Bioremediation Home Remedy Program uses and addresses the three pillars of sustainability: incorporating the importance of community education and empowerment, providing economic support and resources, and keeping the ecological environment a priority. SEEDS is funded by workshop tuition fees and a steady stream of small and large private donations such as Freidman and Bruye Inc.'s offer to conduct soil sampling for free. SEEDS has also received several small grants and developed earned income products and services. Continued help from volunteers and a pulsing Puget Sound community are providing the momentum for SEEDS to continue to promote educational experiences that make lasting changes to better the global community.

Financial Statement

Once we started working with organizations to get the materials we needed, it became less expensive than originally suggested. However, monies were needed to fund consults with remediation specialists and an increase in travel costs also occurred.

SEEDS Bioremediation Home Remedy Program Budget Spent

	Cost
On-site work	
55 gallon rain barrels (3)	$165.00
Drip Irrigation Equipment	$200.00
Shovel	$25.00
Pipe Cutter	$15.00
Chicken Wire	$65.00
Stakes and used wood material	$65.00
Bags	$6.00
String and sampling tools	$20.00
Remediation organisms	
Mycogrow soluble 12ounces	$74.00
BioKlean Culture	$225.00
Mustard Seeds	$6.00
Other associated costs	
Transportation	$500.00
Donated Items	
Mechanized tiller	
Soil Testing	
Biochar	
Total	$1,346.00

SEEDS Bioremediation Home Remedy Program Budget for Future Spending

	Cost
Remediation organisms	
BioKlean Culture	$514.00
Weed Whipper	$75.00
Other associated costs	
Plant Tissue Assessment	$2,100.00
Transportation	$500.00
Total	$3,189.00

Total Amount Projected	**$4,535.00**
Extra Provided	**$1,250.00**

The extra provided will likely be used toward education programming and unforeseen costs.
Following is the current goals and objectives and the updated study methodology and the evaluation components.

Bioremediation Home Remedy Program: Goals and Objectives

SEEDS envisions a permaculture “make-over” of bioremediated garden spaces that have been largely abandoned through concerns about contamination. The Home Remedy Program aims to provide a proven, replicable, and affordable model for remediating soils contaminated with arsenic and lead. Bioremediation techniques are inexpensive and holistic, providing healthy living systems for daily life and food production.

Our goal for the Home Remedy Program continues to be to demonstrate that contaminated soils within the smelter plume area of Vashon can be remediated through ecological and accessible low-technology means in order to provide safe food production, according to the rigorous standards of Washington State, which are considerably more stringent than the EPA standards. The objectives of this particular study have evolved to be ecologically sound as well as obtainable goals for an initial study of this size. Now the objective is to compare effectiveness between three combinations of different ecological methods: funguses, micro-organisms and mustard plants, rather than ferns. The The different combinations will allow us to study these methods’ effectiveness when working together, to view their effectiveness in a “guild.” A guild is a permaculture term meaning “harmonious assembly of species (Mollison, 60)”.

These test sites will be transformed into sites for on-going community education in both bioremediation and permaculture approaches, providing a positive and lasting impact not only on the health of Vashon Island’s 13,000 residents but on other communities facing similar challenges. The program will also promote food sovereignty, which means not merely food security (which might be obtained through food transported long distances), but the maximizing and democratizing of local resources for food production.

Study Design

The Bioremediation Home Remedy initial study has evolved since the grant proposal was funded. After the proposal was sent to the Harris and Frances Block Foundation, more research revealed the invasiveness of the fern species chosen to use for the phytoremediation component. Another plant, Brown Mustard (*Brassicaea juncea*), has been revealed as a well known hyperaccumulator of both arsenic and lead. The initial study has also been simplified to only test different guilds rather than one species at a time. If one of the guilds is successful, then further research will look at the plants, microorganisms, and fungus separately to see if only one of the components is needed for a successful remediation. We hypothesized that there would be negligible differences between the low and high technology methods of bioremediation, and thus combined the methods for a doable, affordable, simplified research design. We also hypothesized that the guild bioremediation plots would be more effective at remediating the soil than the individual specie and species plots. Therefore, by studying the guilds first we can more easily afford soil testing in the initial stages of the research as we build more partnerships that will enable further funding of the project at a more complex level of research. The guilds are all focused toward phytoremediation, the process of plant accumulation of toxins, aiding in the acceleration and efficiency of this process.

Methodology

Due to the changes made to the study design the methods have changed as well. SEEDS researchers will now start with 16 one meter square plots (rather than 18) and 2 control plots (instead of 1). Instead of using specific mushrooms such as Shaggy mane which requires close monitoring when fruiting bodies emerge, an all around mycelium will be applied for inoculating the soils. We will use mustard seed (*Brassicacea juncea*) which has been studied for successful arsenic and lead remediation. Biochar is a new addition to the research design and will be used to house mycelium and microorganisms. Each plot will have one of four combinations with the overall all goal of an efficient and affective phytoremediaiton. Four plots will have a guild of brown mustard (*Brassicaea juncea*), mycelium to increase plant uptake, and a blend of microorganisms called BioKlean. Four plots will have mustard seed, mycelium, and biochar. Another four plots will have mustard seed, BioKlean and biochar. The last four plots will have mustard seed, mycelium, BioKlean, and biochar. Low technology methods will be used as each plot will be hand tilled and hand seeded while the high technology methods will be kept using a specialty blend of BioKleen probiotics, a drip irrigation system, and purchased mycelium.

The study will be conducted over an initial six month period starting May 12th, 2011to allow for a full growing season to be completed. Also, as a part of the research process, various education programs are happening. One permaculture hands on lesson occurred May 8th during a soils workshop that focused on remediation using these methods. Other workshops will bring hands on education to the site. One researcher is conducting her thesis work at the site while other SEEDS researchers are gaining wisdom in different approaches as well. By the fall a culminating public workshop will take place that highlights the bioremediation program within the framework of "Radical Sustainability," a vision SEEDS has adopted from Scott Kellogg and Stacy Pettigrew of the Rhizome Collective. Radical Sustainability champions the necessity that resources required for meeting people's needs be designed, built, maintained, and controlled by those who use them, and materials be likewise accessible, low-cost, and shared. The SEEDS Home Remedy Program works toward this vision. The Home Remedy Program is also featured in an educational art exhibit at a local skate, frisbee and bike park dramatizing the processes and benefits of bioremediation, designed by noted eco-artist Beverly Naidus.

Analysis

Initial testing has been done with a Thermo Scientific Niton® XRF analyzer to conduct Positive Material Identification (PMI) on site to locate where the most contamination occurs within the sites. 36 initial soil samples are being analyzed by Friedman & Bruya, Inc. Environmental Chemists for arsenic and lead levels testing on a Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with a concluding sample set to be tested in the late fall. Plant tissue will also be tested twice for arsenic and lead, once for each plant harvest. The analysis procedure will consist of the following steps:

- We will take six sub-samples from each plot for initial testing with three taken randomly at 2inches and three taken at 6 inches deep. Each sample will be sieved and grinded down with mortar and pestle according to the EPA's procedural guidelines. Then the three will be from the same plot and depth will be mixed together and sub-sampled three times, repeating this for each plot's group. The sub-samples will be compacted into a small testing container and analyzed three times for an average. We will conduct this soil analysis at the beginning and end of the six months. One set of plots will be analyzed each month.
- *Brassicaea juncea* will be harvested after 60 days before the plant goes to seed. One plot from each set will leave the plants to seed. Each plant from its specific site will be dehydrated and powdered, then mixed with samples from its plot and sub-sampled three times. Each sub-sample will be compacted into a small testing container and analyzed three times for an average with the Niton XRF, with five percent of the samples sent to Friedman and Bruya, Inc.
- The categorical data sets will be analyzed with a Chi-squared analysis to insure statistical significance. Our categorical independent variables are each plot, while our dependent variables will be the fruiting mushroom bodies, aerial portions of the ferns and the control plot. The before and after levels of lead and arsenic in each plot will then be compared to the control plots using T-tests which is typically used to compare the before and after samples. In order to observe the changes that occur over time, measurements taken each month will be plotted on an x-y scatter plot to show changes in concentration.
- We will conduct Analysis of Variance (ANOVA) which compares three or more means in order to find the most efficient process.

Evaluation

The Bioremediation Home Remedy Program will be evaluated on three main principles.

The first criteria for evaluation will be the ability of the bioremediation process to successfully clean the contaminated soils, confirmed by testing of the soil and aerial bodies of the mustard plants. We are just now (May 11th, 2011) planting our first round of plants and sending off our first samples. More time is necessary to see whether this process is successful.

The second component will be the collection of scientific and social data to gain a better understanding of bioremediation processes and interactions between species. This will give residents, as well as local, state and federal agencies better tools for remediating contaminated soils. We have come a long way in our understanding of Bioremediation and are successfully sharing it with different groups through workshops and conversations at a more personal level. Data has been collected and more is on the way!

The last important principle to be evaluated will be the level of community involvement and education. This will be assessed by the attendance at three public workshops held by SEEDS at the bioremediation sites and by the community's feedback and education about the study, including articles in the local newspaper, outreach at the local farmer's market and other venues, and updates on the SEEDS' website. We plan to have bigger events focused toward this project in particular and have held smaller gatherings with Home Remedy as one of the main topics. Further evaluation will be done as the Home Remedy Program is put into practice throughout the community.

Project Description and Timeline
Background

History and Mission of SEEDS

Headquartered on Vashon Island, the Social Ecology Education and Demonstration School (SEEDS) works to meet the urgent need for an educational ecological project aimed at both local and global communities. The mission of SEEDS is to develop and offer educational experiences that enhance people's abilities to knowledgeably and creatively address the interwoven social and ecological crisis of our time. Through an intensive and interdisciplinary study, participants gain a critical and comprehensive understanding of social and ecological reconstruction. SEEDS provides participants with opportunities to test various environmental reconstructive strategies by means of individually designed practicum learning experiences.

Beginning last year, SEEDS has played a key role in the development of "Vision for Vashon." A comprehensive community development effort, Vision for Vashon sparked citizen work groups in food security and grassroots sustainability projects. SEEDS' goals in relation to food sovereignty include the promotion of collaborative farming/gardening, and education in permaculture approaches. Through SEEDS the following research project is being proposed as a permaculture bioremediation process to alleviate heavy metals in soils.

Heavy Metals on Vashon

Smelting plants have caused long lasting impacts on surrounding environments. Even after smelting plant closings, heavy metals are left behind in soils causing contamination and toxicity. Vashon and Maury Islands of the South Salish Sea presently have high levels of arsenic and lead in the soils due to the American Smelting And Refining Company *(*ASARCO) smelting plant, operated from 1887 to 1986 in Ruston of Tacoma, Washington (Glass, 2003). The southern half of Vashon Island and all of Maury Island were under the smelter plume windfall area, leaving the areas contaminated with large amounts of the heavy metals, arsenic and lead. These metals impact the residents' ability to grow food for personal and market consumption due to the detrimental impact they have on human health when inhaled or absorbed through the skin. Soil remediation is a necessity for the Vashon community in order to enable reliable, healthy and local food sourcing.

While the ASARCO plant was designated a Superfund site in 1983 by the EPA. Under Superfund regulations the EPA has documented the contamination of lead and arsenic in the adjacent areas (EPA, 2009). The Washington State Department of Ecology (WSDOE) found concentrations of 460 parts per million (ppm) of arsenic and 1300 ppm of lead on certain soils on the south side of Vashon Island (Glass, 2003). This level of contamination is well over the safe limit for bare soils and is more than three times the limit for areas with children (Glass, 2003). These levels are far above Washington State's limit of 20 ppm concentration standard for arsenic and the state's 250 ppm for lead (WSDOE). See Table 1.

Soils contaminated with heavy metals such as lead and arsenic can be dangerous to humans and may be ingested, inhaled or absorbed through the skin. Simply being outside and tracking the contaminated soil into the house on shoes and clothes is a means of ingestion. Growing vegetables in home gardens is also a way in which heavy metals enter the body (Department of Ecology State of Washington, 2010). Excessive exposure to arsenic can cause lung, bladder and other cancers to humans (Stamets, 2005). Lead exposure affects bones, reproductive and nervous systems and has been shown to be detrimental to the developing human brain.

Bioremediation

Bioremediation is the decontamination method used on sites utilizing organisms, namely plants, fungi, and microorganisms. There are several advantages to bioremediation methods versus other forms of remediation. Bioremediation actually transforms the contamination, such as lead or arsenic, and even radionuclides such as uranium compounds, present in the soil into something that is less toxic (Iwamoto and Nasu, 2001). More traditional methods of remediation simply remove the contaminated soil and deposit it in another location, and does not address the toxicity leaving it for future generations. Bioremediation practices also tend to be low cost, when compared to other methods for remediation (Lloyd and Renshaw, 2005). Another advantage for bioremediation is the multiple organisms that can be used for bioremediation, each with their own strengths.

In our study, we will be using compost tea and a microbrial cocktail called BioKlean, mycorrhizae and Shaggy Parasol for the fungi reassimilation and accumulation of arsenic and lead, and mustard plants for hyperaccumulation. We will be looking at low and high cost methods. All of the research will be done in back yards of Vashon Residents and in Public Parks.

The Evergreen Component

The Evergreen component of the SEEDS Bioremediation Home Remedy Program will provide a pivotal piece to the overall goal of the project by having current undergraduate students in the Environmental Analysis class help me conduct soil sampling on Vashon Island and soil testing for arsenic and lead on the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at the Evergreen Campus this spring quarter. Then we will able to set up a statistical baseline of contamination levels for the concurrent readings to be compared with. The project will include a soil collecting field trip in early April (or possibly even late march) to Vashon where samples will be collected from each test plot. On Vashon, they will be shown various projects being implemented by SEEDS while we have open discussions about various social ecology issues occurring within the community. After the soil samples are taken, analysis on the samples will be done the following weeks in April. Then the results will be mapped and statistically analyzed (in order to find variance) to be presented in the EA class in June as the quarter's final presentation. The students will also get the opportunity to participate in a permaculture class being held at the site on May 8th for the Soil Education Day in the Spiral Visions Social Ecology Education and Demonstration School (SEEDS) Permaculture Design Course. The overall Bioremediation Home Remedy Project will be concluded in one to two years from now when the site will be evolved into a permaculture food garden. The funding request being asked for the Student Foundation Activity Grants is for the soil testing and soil sampling trip only.

Product
There will be many end products to the Bioremediation Home Remedy Project, but specific to the Evergreen component of the project students will be provided with the opportunity to gain field and lab experience working on a project that benefits the larger community. They will be contributing to my thesis work, important remediation research, as well as community health and awareness. Products will include learning experience, documented scientific analysis of these contaminated soils, and a thesis paper. A baseline reading of the levels of arsenic and lead in the soils at this site is vital in understanding the viability of the different remediation techniques being studied. Student's will be able to continue their participation in this research and become involved at many levels in the 1-3 year process of turning a polluted, hazardous site into a permaculture garden.

Current Academic Work
I am currently a student in Evergreen's Masters of Environmental Studies (MES) Program beginning work on my thesis. I have been working with SEEDS in contracts and through volunteer work for the past year in order to fund and implement the Bioremediation Home Remedy Project. I have done extensive research, alongside Evergreen students LaDena Stamets and Austin Walsworth, on some of the most efficient remediation techniques dealing with heavy metals. The proposed project as outlined in the background section of this request, will be focused on using different microbrial cultures, fungal, and plant based remediation in combinations and alone in high and low technology plots. I have been contracting with SEEDS to do research in the arts, social ecology, and various food system ideas.

Importance to My Evergreen Career
Receiving funding for the soil sampling and testing while providing students with scientific and community building experiences will greatly impact the ability of my thesis work to be conducted. The cost of having a science lab do the testing is very expensive, around $18,000, and does not bring this opportunity to science students who have been working in the EA class. Receiving funding for the soil testing is pivotal, but providing hands on experience for students is an extra benefit for all involved.

In line with Evergreen's Teaching and Learning Values
This is an interdisciplinary study enabling scientific procedures to overlap and commingle with real world problems working towards real world solutions. Bringing students into this process using their scientific skills on the ICP-MS in relation to remediation and permaculture practices allows these skills to be involved in contributing to the mitigation of the toxicity of heavy metals in people's homes and back yards. This is also a collaborative process bringing many students together to focus on one issue where they can share their experiences, skills and knowledge. This project also allows the students to practice their own beliefs and judgments on the best ways to deal with situations inside and outside of the lab in relation to the bioremediation research and, mainly, the intricacies of soil testing. By having these students participate in my research,

they will help me to apply abstract theories in my project and will allow me the benefit to participate in the real testing of the soils.

Significance

Funding the testing of these soils is significant in my personal research and in the research of the remediation community at large. Furthermore, it will significantly impact those students who are able to participate in the specifics of the soil testing bringing them into a bigger focus of what those soils are a part of; a community working to grow their own food, the EPA trying to find viably and cost effective methods to deal with the damage left from the industrial revolution, and the Evergreen community being able to support this process. I am ready to get dirty and implement my thesis design and methods with some real hands on work!

The Evergreen State College Student Activities Fund

Bioremediation Evergreen Soil Testing Budget			
Cost Items and Supplies	**Quantity**	**Price/Unit**	**Cost**
polyethylene 50mL test tubes	1000tubes	3.75/25 tubes	$150.00
polyethylene 25mL test tubes	1000tubes	7.00/50 tubes	$140.00
trace metal grade nitric acid (1M HNO3)	125mL	185/125mL	$185.00
concentrated nitric acid (16M HNO3)	5mL/sample	33.25/2.5L	$33.25
ultra high purity grade (> 99.99%) germanium (Ge) standa	125mL	77/125mL	$77.00
ultra high purity grade (> 99.99%) Thulium (Tu) standard	125mL	40/125mL	$40.00
ultra high purity grade (> 99.99%) lead (Pb) standard	125mL	35/125mL	$35.00
ultra high purity grade (> 99.99%) arsenic (As) standard	125mL	35/125mL	$35.00
H2O2 (30%)	32oz	66.44/32oz	$66.44
NIST SRM 2710 Montana Soil	100g	210/100g	$210.00
Whatman No .41 Filter paper	300	18.54/100	$55.62
pvc piping for core sampling	5 Ft	.41/ft	$2.05
USS #10 Sieve	1	34.00/seive	$34.00
Gloves – medium	2 boxes	7.25	$14.50
Gas and Ferry Fare for 3 trips	3 trips	55/trip	$165.00
unforseen costs			$100.00
		Total Cost:	**$1,242.86**
*feild work for this project has recieved funding from the Harris and Franics Block Foundation recieving funds around $5,000. Soil testing costs are costs that still need to be covered.			
**if there are any questions or thoughts about partial funding please contact Shannon Clay			