Phytoextraction and Accumulation of Mercury in Selected Plant Species Grown in Soil Contaminated with Different Mercury Compounds

Y. Su, F. Han, S. Shiyab, D.L. Monts
Institute for Clean Energy Technology (ICET)
Mississippi State University
205 Research Blvd, Starkville, MS 39759, USA

ABSTRACT

The objective of our research is to screen and search for suitable plant species for phytoremediation of mercury-contaminated soil. Currently our effort is specifically focused on mercury removal from the U.S. Department of Energy's (DOE) Oak Ridge Site, where mercury contamination is a major concern in the Y-12 Watershed area. In order to cost effectively implement those remediation efforts currently planned for FY09, it is necessary now to obtain an improved understanding of biological means of removing mercury and mercury compounds from the Oak Ridge ecosystem. Phytoremediation is a technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. In particular, phytoextraction is the uptake of contaminants by plant roots and translocation within the plants to shoots or leaves. Contaminants are generally removed by harvesting the plants. We have investigated phytoextraction of mercury from contaminated soil by using some of the known metal accumulating wild plants since no natural plant species with mercury hyperaccumulating properties has yet been identified. Different natural plant species have been studied for mercury uptake, accumulation, toxicity and overall mercury removal efficiency. Various mercury compounds, such as HgS, $HgCl_2$ and $Hg(NO_3)_2$, were used as contaminant sources. Different types of soil were examined and chosen for phytoremediation experiments.

We have applied microscopy and diffuse reflectance spectrometry as well as conventional analytical chemistry to monitor the phytoremediation processes of mercury uptake, translocation and accumulation; and the physiological impact of mercury contaminants on selected plant species. Our results indicate that certain plant species, such as beard grass (*Polypogon monospeliensis*), accumulated a very limited amount of mercury in the shoots (<65 mg/kg), even though root mercury accumulation is significant (maximum 2298 mg/kg). Consequently, this plant species may not be suitable for mercury phytoremediation. Other plant species, such as Indian mustard (*Brassica juncea*), a well-studied metal accumulator, exhibited severe chlorosis symptoms during some experiments. Among all the plant species studied, Chinese brake fern (*Pteris vittata*) accumulated significant amount of mercury in both roots and shoots and hence may be considered as a potential candidate for mercury phytoextraction. During one experiment, brake ferns accumulated 540 mg/kg and 1469 mg/kg in shoots after 18 days of growing in soils treated with 500 ppm and 1000 ppm $HgCl_2$ powder, respectively; no visual stress symptoms were observed. We also studied mercury phytoremediation using aged soils that contaminated HgS, $HgCl_2$, and $Hg(NO_3)_2$. We have found that up to hundreds of ppm mercury can be accumulated in the roots of Indian mustard plants grown with soil contaminated by mercury sulfide; HgS is assumed to be the most stable and also the predominant mercury form in Oak Ridge floodplain soils. We have also started to investigate different mercury uptake mechanisms, such as root uptake of soil contaminant and foliar mercury accumulation from ambient air.

INTRODUCTION

Mercury is a non-nutritive heavy metal that poses significant environmental and health concerns. Hg is one of the rare elements. Its content in the earth's crust is estimated to be $5x10^{-5}$ % and it is 62nd in abundance among the elements. However, the World Health Organization (WHO) has estimated that each year 10,000 tons of mercury is released globally from both natural and anthropogenic sources. Naturally occurring Hg is released by degassing of the earth's crust, from volcanoes, and from evaporation of oceans [1]. Industrial age anthropogenic mercury is estimated to be 640,000 tons [2]. Mercury has a wide variety of uses in industry, medicine, dentistry, batteries, science, and military applications. The burning of fossil fuels and medical waste incineration accounts for more than 80% of all anthropogenic mercury [3]. Fifty-five percent of the total consumption of mercury is by chloralkali synthesis (used in electrodes), the wood pulping industry, paint, and electrical equipment.

Boening [1] recently reviewed the ecological effect, transport, and fate of mercury. Most of the mercury that has been released by anthropogenic sources is currently retained in surface soils as complexes of ionic mercury, Hg(II), bound with sulfides, clay particles, or organic matter. The stability of Hg(II) in soils gives it an estimated mean soil residence time of at least 1000 years. As a result of past and current mercury releases, there are a growing number of dangerously contaminated sites that will remain environmental hazards for thousands of years unless remediated [3].

The Oak Ridge Reservation of the U.S Department of Energy (DOE ORR) is a Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) site that is on the U.S. Environmental Protection Agency (EPA)'s national priorities list (NPL). It is being cleaned up under a federal facilities agreement with US EPA and the State of Tennessee. Historically as part of its national security mission, the U.S. Department of Energy's Y-12 National Security Facility in Oak Ridge, TN acquired a significant fraction of the world's supply of elemental mercury. The description of the Oak Ridge mercury project can be found on the Internet at http://newweb.ead.anl.gov/techcon/Projects/mercury/description/. According to this website, mercury contamination is ubiquitous in the Y-12 watershed and has been identified as a contaminant in soil, sediment, surface water, ground water, buildings, drains, and sumps.

Mercury-contaminated soils and sediments are commonly remediated by soil excavation, relocation, and burial; soil washing with halogenated substances; heating soil to high temperature; etc. But a majority of these technologies are costly to implement and cause further disturbance to the already damaged environment. Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. Phytoremediation could cost-effectively replace traditional mercury remediation strategies [4].

At sites with significant levels of mercury contamination, such as DOE's Oak Ridge site, it might be desirable to deploy plants that accumulate high mercury loads in harvestable tissues. Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants. This concentration technology leaves a much smaller mass to be disposed. There is some evidence that certain plant species

have the ability to extract and accumulate mercury both from the atmospheric and soil sources [3]. Huckabee *et al*. [5] monitored levels of mercury in vegetation in the vicinity of the mercury mine at Almaden, Spain. Currently, however, no plant species with mercury hyperaccumulating properties has been identified. Patra and Sharma [6] reviewed mercury toxicity in plants. All physiological and biochemical processes in plants may be negatively affected by mercury when plants are exposed to mercury-contaminated soil, water or air.

Gaseous emission of mercury from plants and foliar uptake of atmopheric mercury have long been observed and studied [7-9]. Suszcynsky and Shann [10] studied the accumulation, toxicity response, and Hg distribution in tobacco plants exposed to elemental mercury vapor in a specially designed chamber system and in plants root-exposed to ionic mercury. Plants exposed to elemental mercury vapor accumulated mercury in the shoots with no movement to roots (by day ten). Root-exposed plants showed accumulation of mercury in the roots with movement to shoots by day ten [10].

In a bench scale study conducted at Argonne National Laboratory West, it was found that hybrid willows had the best recovery of mercury in sand experiments and that the recovered mercury was found almost exclusively in the roots [available on the Internet at http://www.bigisland.ttclients.com/frtr/00000160.html]. Rhizosphere bacteria are reported to increase the efficiency of mercury phytoremediation by promoting the accumulation of Hg in tissues of two wetland plants, salt marsh bulrush and rabbitfoot grass [11]. Henry published "An Overview of the Phytoremediation of Lead and Mercury" in 2000 [12].

At the Institute for Clean Energy Technology (ICET), Mississippi State University, a multidisciplinary team of scientists has been studying the impact of heavy metals and radionuclides on the physiological status of selected plant species, and also the application of phytoremediation. The objective of our research is to search for suitable plant species for phytoextraction of mercury-contaminated soil. In this study, we focus on phytoextraction of mercury from contaminated soil by natural plant species. Since there is no known mercury hyperaccumulator, three natural plant species were evaluated for efficiency of uptake of mercury. The three plant species are: Indian mustard (*Brassica juncea*), Beard grass (*Polypogon monospeliensis*), and Chinese brake fern (*Pteris vittata).* Indian mustard has been identified as a high biomass-producing plant with the capacity to accumulate Zn and Cd at higher concentrations in plant cells. Some researchers speculate that if the amount of phytochelatins within a metal-accumulating plant (i.e., some mustard species) could be increased, the level of contaminant removed by plants would also increase. Hg is the third member of the Group IIb triad of the periodic table of elements along with zinc and cadmium. We have previously studied the anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard [13]; and also the phytoavailability and toxicity of trivalent and hexavalent chromium to the same plant species [14]. Chinese brake fern (*Pteris vittata*), a recently recognized hyperaccumulator plant, has been found to extract very high concentrations of arsenic from arsenic-contaminated soil [15,16]. We have recently reported our study of the phytotoxicity and phytoaccumulation of trivalent and hexavalent chromium in brake fern [17]. Beard grass, also known as rabbitfoot grass, has been reported to accumulate up to 13 µg/g dry weight (DW) in shoots during a hydroponic study with 1 mg Hg L^{-1} in the solution [11]. Lenka and coworkers reported that two grass species have been found growing at abandoned solid-waste dumpsite that contained mercury levels as high as 557 mg/kg soil [18]. In our study, two sets of experiments were

conducted to evaluate the phytoremediation potential of these three plant species: a pot study with potting mix where mercury was provided daily as $HgCl_2$ solution; and another separate set of experiments with $HgCl_2$ powder used to spike soil obtained from a local farm. To investigate the effect of mercury accumulation on leaf structure, leaf samples from every group were collected for microscopic study on the last day of the soil study with Chinese brake fern. Spectral reflectance was also used to monitor the plant physiological status throughout the soil phytoremediation experiment. A third experiment was designed to investigate the bioavailability and uptake of mercury contaminants in aged soil with different mercury sources.

MATERIALS AND METHODS

Chinese brake ferns (*Pteris vittata)*, 4-5 months old, were obtained from Edenspace Systems (Edenspace Inc., Dulles, VA). Mustard seeds were a commercial variety obtained locally. The seeds were sown in plastic pots containing potting mix. The mustard plants were grown to about six weeks old before being exposed to mercury contamination. Beard grass (*Polypogon monspeliensis*) seeds were obtained from Pinetree Garden Seeds (New Gloucester, ME). The grass was used for phytoremediation experiments after germinating in potting mix soils and growing for about eight weeks.

Pot Study with Potting Mix

The first set of pot study was done with Miracle-Gro Potting Mix (Miracle-Gro Lawn Products Inc., Marysville, OH). Plants were all grown in plastic pots containing potting mix. Mercury was supplied as $HgCl_2$ solutions. Indian mustard plants and beard grass were grown in pot containing 2.0 kg of potting mix; while Chinese brake ferns were grown in smaller pots with 1.0 kg of potting mix. The mercury treatment was started at the same day for all three plant species. For $HgCl_2$ solution, 100 ml pot^{-1} day^{-1} was provided to Indian mustard plants, while 50 ml pot^{-1} day^{-1} of $HgCl_2$ solution was provided to brake ferns and beard grass. Mercury treatments with five replicates in each group were supplied with 100 ppm (PMT1), or with 500 ppm (PMT2) $HgCl_2$ solution. All the treatment groups, along with the control (T0), were arranged in a completely randomized design. The mercury treatments were supplemented with nutrient solution to avoid any water or nutrient deficiencies. The composition of Modified Hoaglands Nutrient solution [19] is: 0.5 mM $Ca(NO_3)_2$, 3.1 mM NH_4NO_3, 0.01 mM KH_2PO_4, 50.0 μM KCl, 0.2 μM $CuSO_4$, 12.0 μM H_3BO_4, 0.1 μM $NiSO_4 \cdot 6H_2O$, 2.0 μM $MnSO_4 \cdot H_2O$, 0.5 μM $ZnSO_4 \cdot 7H_2O$, and 0.2 mM $MgSO_4$. The plants were kept outdoors in an enclosed area except during extreme weather conditions.

All the mustard groups were harvested after three weeks of mercury treatment; all the beard grass and bake fern groups were harvested after four weeks of mercury treatment. Roots were washed with distilled water. Both roots and shoots were dried at 80° C in an oven for 48 hours and then prepared for chemical analyses.

Pot Study using Soil Spiked with $HgCl_2$ Powder

The second set of experiments was performed with mercury chloride-spiked soil. The soil is an Ora soil (fine-loamy, siliceous, thermic Typic Fragiudults) obtained from local farmland near Starkville, located in the Upper Coastal Plain of Mississippi. The soil was formed in weathered,

stratified deposits of sand, silt, clay and gravel in several geological formations. The soil exhibits mature pedogenic development. Surface soil (0 to 15 cm) was sampled, air-dried and ground to pass a 2-mm sieve. Relevant soil properties are presented in Table I. About 1.5 kg of air-dried soil was weighed and transferred into 6-inch (15 cm) diameter plastic pots. Nitrogen, phosphorous, potassium (N : P : K = 1 : 1 : 1) were added to soils as base fertilizers. $HgCl_2$ was added as a powder to the soil at concentrations of 250, 500 and 1000 mg/kg. After mixing in $HgCl_2$ (by using a home-made V tumbler), water was added to the soil to the field-capacity regime.

Table I. Selected properties of Ora soil (fine-loamy, siliceous, thermic Typic Fragiudults) from Starkville, Mississippi, USA.

Soil Properties	**Unit**	
pH		4.41
Cation Exchange Capacity (CEC)	$cmol_c\ kg^{-1}$	7.82
Hygroscopicity	H_2O%	3.1
Texture		Silt Loam
Sand	%	32
Silt	%	62.9
Clay	%	5.1

Four- to five- month old Chinese brake ferns (*Pteris vittata)*, obtained from Edenspace Systems (Edenspace Inc., Dulles, VA), were transferred to pots filed with freshly spiked soil. All the treatment groups along with the control (T0) were arranged in a completely randomized design. Each group consisted of five replicates. The ferns were harvested after grown in mercury-spiked soil for 18 days. Roots were washed with distilled water. Both roots and shoots were dried at 80° C in an oven for 48 hours and then prepared for chemical analyses.

After the experiment with brake ferns, soils of the same group were re-mixed and used for a phytoremediation study with beard grass. Beard grass (*Polypogon monspeliensis*) seeds were obtained from Pinetree Garden Seeds (New Gloucester, ME). The grass was used for phytoremediation experiments after germinating in potting mix soils and growing for about eight weeks. All the treatment groups along with the control (T0) were arranged in a completely randomized design. Each group consisted of five replicates. The ferns were harvested after growing in mercury-spiked soil for three weeks. Both roots and shoots were dried at 80° C in an oven for 48 hours and then prepared for chemical analyses.

Pot Study using Aged Soil with Different Mercury Contaminant Sources

A soil was collected from a private farm in Roane County, Tennessee, near Oak Ridge's East Tennessee Technology Park (ETTP) where the K-25 facility was located. Soil was air-dried and homogenized through a 2-mm sieve. Mercury compounds were originally added as powder in the forms of $Hg(NO_3)_2$ at 100 mg/kg; $HgCl_2$ at 100, 250 and 1000 mg/kg initially (May, 2004); and HgS at 1000 and 2000 mg/kg. About 1.5 kg of prepared soil was weighed and transferred into 6-

inch (15-cm) diameter plastic pots. This set of soils was used for a series of mercury phytoremediation experiments; hence the total soil mercury concentration and most importantly the bioavailable portion of the mercury contaminant have been changed. During the spring of 2006, the well-aged soil was remixed within each group and used for a phytoremediation experiment with Indian mustard. Plants of seven-week old (early spring) were transferred to the aforementioned soil, and were grown for 54 days before harvest. Both roots and shoots were dried at 80° C in an oven for 48 hours and then prepared for chemical analyses.

Chemical Analysis

Plant samples (approximately 0.5 g) were digested with concentrated HNO_3 and H_2O_2, and soil samples were digested with 4M HNO_3. Mercury content in plant shoots, roots and soil were analyzed using two methods: ICP-AES (inductively coupled plasma-atomic emission spectrometry) and CVAAS (cold vapor atomic absorption spectrometry). An Optima 4300 DV ICP-AES equipped with a Scott-Flow nebulizer, an ELAN 9000 ICP-MS, and a FIMS 100 CVAAS, all from Perkin Elmer Instruments, were used to detect Hg concentrations in solutions.

RESULTS AND DISCUSSION

Potting Mix Study

Mercury accumulations by the three plant species after three to four weeks of mercury solution treatment are shown in Figure 1. As we can see from Figure 1, all three plant species accumulated mercury in both plant shoots and roots, with the majority of mercury accumulation being found in the roots. Even though the maximum values of shoot mercury accumulation of the three plant species were comparable, mercury-treated Indian mustard and beard grass exhibited severe stress. Chlorosis was observed for all mercury-treated Indian mustard and beard grass groups, with the mustard T2 (500 ppm) group showing the strongest symptoms. The mustard T1 and T2 groups had to be harvested one week earlier because of severe stress. Chlorosis was not visually observed for the two mercury-treated fern groups during the treatment process, despite the fact that ferns accumulated approximately twice as much mercury in roots when compared with the respective groups of Indian mustard and beard grass.

We observed a very noteworthy fact during the potting mix study. Mercury accumulation was found in plant tissues for all three T0 (control) groups. Average mercury accumulations for the fern T0 group are 10.51±1.46 (mg/kg) for shoots and 6.45±0.65 (mg/kg) for roots. For the Indian mustard T0 group, average mercury accumulations are 6.04±1.80 (mg/kg) for shoots and 0.42±0.24 (mg/kg) for roots. And for the beard grass T0 group, average mercury accumulations are 5.61±1.07 (mg/kg) for leaves and 1.53±0.48 (mg/kg) for roots. Since no mercury solution was provided to any of the T0 groups, we assume that the observed shoot mercury accumulation resulted from foliage absorption of aerial mercury vapor in the surrounding atmosphere. All the plants were kept in racked containers close to each other inside a chained area of 20' x 20' (6 m x 6 m). As 50 - 100 mL pot^{-1} day^{-1} of $HgCl_2$ solution was provided to the metal-treated plants, mercury concentration in the immediate surrounding atmosphere has to be taken into

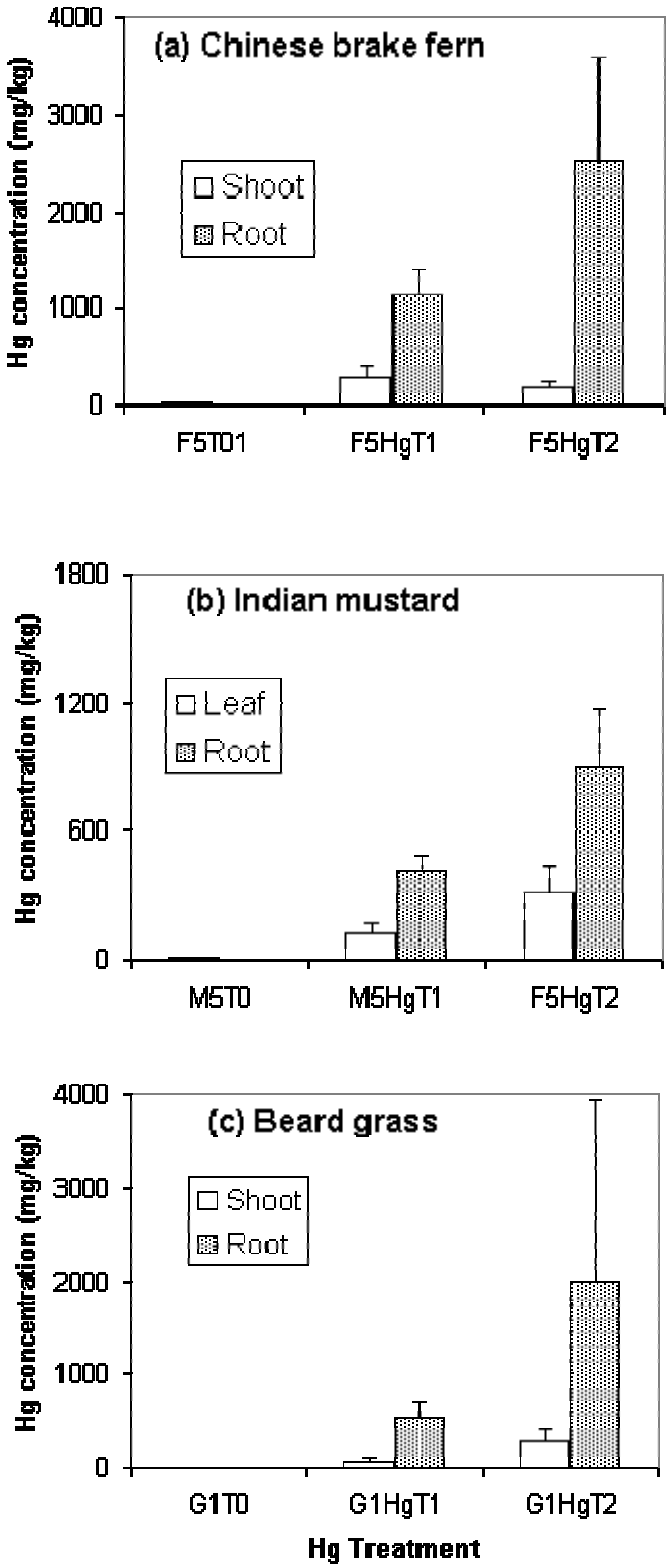

Figure 1. Plant shoot and root mercury accumulations from potting mix pot study (in mg/kg dry weight): (a). Chinese brake fern; (b). Indian mustard; and (c). beard grass.

consideration. As we mentioned in the Introduction section, gaseous emission of mercury from plants and foliar uptake of atmospheric mercury have long been observed and studied [7-10, 20]. For the Indian mustard and beard grass T0 groups, shoot mercury accumulation is 4-6 times higher than root accumulation, which is below 1.07 mg/kg. We believe that this suggests very limited shoot-to-root mercury translocation. For the Chinese brake fern T0 group, average root mercury accumulation was found to be 6.45mg/kg, which is about half of the shoot accumulation. This might have resulted from some level of shoot-to-root mercury translocation. More systematic experimental studies need to be done in order to draw decisive conclusions on this issue. However, this is beyond the scope of the research reported here. Since mercury accumulations among the three T0 groups are significantly lower than the mercury-treated groups, we believe that the foliar absorption is only a minor contribution in overall mercury uptake and accumulation.

Pot Study using Soil Spiked with $HgCl_2$ Powder

Another set of experiments was completed using mercury-spiked Mississippian (MS) soil that was sampled from a private farm near Starkville, MS. $HgCl_2$ was added as powder to the soil in concentrations of 250, 500 and 1000 mg/kg. The prepared soil was first used in a phytoremediation experiment with Chinese brake fern. The soil was then re-mixed within each group and used for a pot-study using beard grass. The chemical results for the fern experiment are summarized in Table II. All data shown in Table II are averages of five replicates. The mercury concentrations in soil listed in Table II are the analytical results after the fern experiment. In addition to plant uptake, leaching may be a major reason that the final soil mercury concentration is significantly lower than the initial value. Atmospheric evaporation may be a minor channel for soil mercury loss.

Table II. Biomass and mercury concentration in Chinese brake fern.

Treatment	Hg in shoots (mg/kg)	Hg in roots (mg/kg)	Shoot biomass (dry weight, g)	Final Hg in soil (mg/kg)
F4HgT0 (Control)	0.38 (0.53) c†	BD	6.1 (1.6)	BD
F4HgT1 (250 mg/kg)	123 (88) b	749 (330) b	5.9 (1.6)	85 (23) c
F4HgT2 (500 mg/kg)	540 (393) b	1525 (786) b	3.9 (1.1)	207 (43) b
F4HgT3 (1000 mg/kg)	1469 (761) a	6802 (3325) a	3.9 (0.3)	413 (77) a

*The numbers in parenthesis indicate standard deviation. #BD - below detection limit.
† Means followed by a different letter are significantly different at the 0.05 probability level, grouped into classes a, b and c

After growing 18 days in mercury-contaminated soil, none of the fern plants showed any visual stress symptoms, such as chlorosis. The highest shoot mercury accumulation is found to be 1469 mg/kg; and the highest root accumulation is 6802 mg/kg. Both shoot and root accumulations are linearly correlated with soil mercury concentration as shown in Figure 2. This indicates high mercury bioavailability in the spiked soil.

As shown in Table II, the biomass (shoot dry weight) of the two groups with higher mercury soil concentrations (F4HgT2 and F4HgT3 groups) are significantly lower than the T0 (control) group. The correlation between biomass and soil mercury concentration is shown in Figure 3. According to Chapin [21], all plants respond to environment stress in basically the same way, through the decline in growth rate and acquisition of nutrient resources. Relative water content (RWC) of shoots was measured gravimetrically and calculated from the formula: RWC (%) = (fresh weight- dry weight)/fresh weight x 100 [22]. The RWC gradually decreases as the soil mercury concentration increases. Losing leaf water is another common symptom related to heavy metal toxicity. Several studies have indicated that visible chlorosis is a common stress response. However, during our experiment, Chinese brake fern accumulated mercury in its shoot at concentrations as high as 1479 mg/kg without visual stress symptoms, such as chlorosis. This indicates that this particular fern species might be a suitable candidate for mercury phytoextraction, especially since no mercury hyperaccumulator has yet been identified.

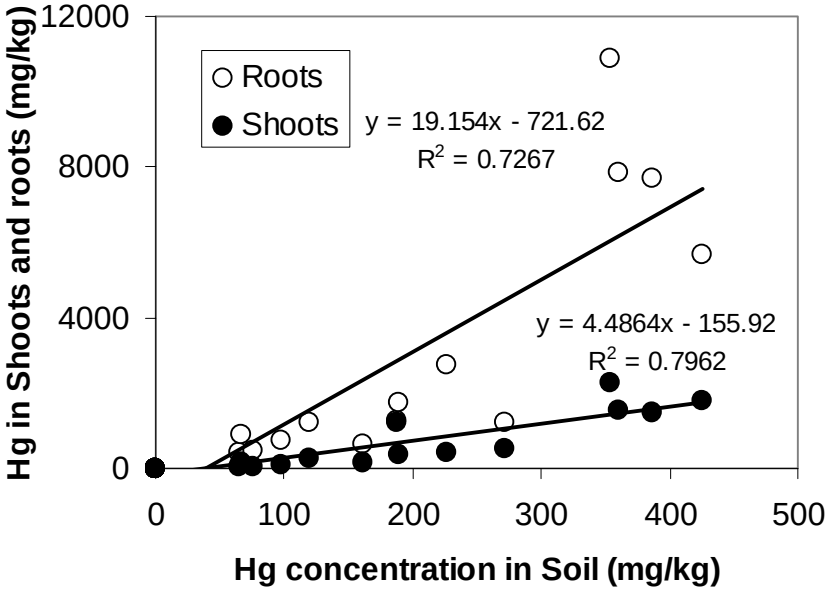

Figure 2. Correlation between shoot and root mercury accumulation with soil mercury concentration for Chinese brake fern from spiked soil study.

After the experiment with Chinese brake fern, the soil from each group was then re-mixed for a pot study using beard grass. The chemical results for the beard experiment are summarized in Table III. The roots of plants from all three beard grass groups grown on mercury-contaminated soil accumulated significant amounts of mercury; after 22 days, the highest root accumulation was by the T3 group, 2298 mg/kg. However, the highest shoot accumulation is only 65 mg/kg, indicating a very poor root-to-shoot translocation rate. From the results of the potting mix study and the soil experiment, it is apparent that beard grass is not a candidate for mercury phytoremediation.

Table III. Mercury concentrations in Beard grass shoots and roots and soil (average with standard deviation).

Treatment	Hg in shoots (mg/kg)	Hg in roots (mg/kg)	Hg in soil (mg/kg)
G5HgT0 (Control)	6.08 (3.89)	9.73 (10.7)	BD
G5HgT1 (250 mg/kg)	40 (27)	1579 (855)	85 (23)
G5HgT2 (500 mg/kg)	26(12)	2241 (1101)	207 (43)
G5HgT3 (1000 mg/kg)	65 (40)	2298 (468)	413 (77)

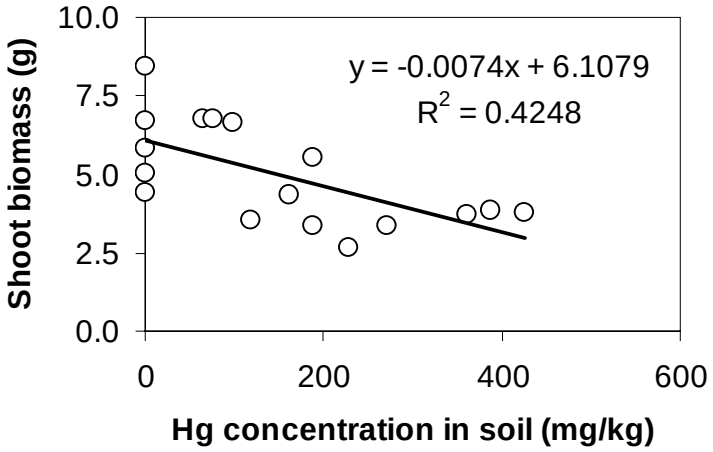

Figure 3. Correlation of shoot dry weight (biomass) with Hg concentrations in soil for Chinese brake fern grown in mercury-contaminated soil.

Pot Study using Aged Soil with Different Mercury Contaminant Sources

The third set of experiments was designed to study the plant uptake of mercury with aged soil originally contaminated by different mercury sources, such as $Hg(NO_3)_2$, $HgCl_2$, and HgS. Indian mustard plants were grown in the aged soils for almost eight weeks before harvest. The shoot and root concentrations are listed in Table IV. As we can see from Table IV, the bioavailability and hence the plant uptake of mercury from these aged soils are limited when compared with freshly spiked soil. However, there are two significant observations from this set of experiments: First, the mercury contaminant from the group with HgS as the original source was uptaken and accumulated in both roots and shoots of Indian mustard plants, with shoot and root mercury concentrations of 79 and 87 mg/kg dry weight, respectively. This is significant because mercury sulfide is assumed to be the most stable and predominant mercury form in many contaminated soils. We are currently conducting systematic experiments to further investigate this phenomenon. Second, mercury accumulation in both shoots and roots of mustard plants grown in aged soil contaminated originally by mercury chloride are significant, reaching levels of 325 and 1775 mg/kg dry weight, respectively. The shoot and root mercury accumulations from the third experiment are shown in Figure 4.

Table IV. Mercury concentrations in shoots and roots of Indian mustard grown in aged soils contaminated by $Hg(NO_3)_2$, $HgCl_2$, and HgS (average with standard deviation).

Treatment	Hg in shoots	Hg in roots	Original Hg in soil
	mg/kg	mg/kg	mg/kg
$Hg(NO_3)_2$	2.1 (2.5)	24 (17)	100
$HgCl_2$ -1	0.8 (0.8)	26 (11)	100
$HgCl_2$ -2	12 (22)	110 (39)	250
$HgCl_2$ -3	325 (287)	1775 (1096)	1000
HgS -1	35 (29)	17 (11)	1000
HgS -2	79 (51)	87 (43)	2000

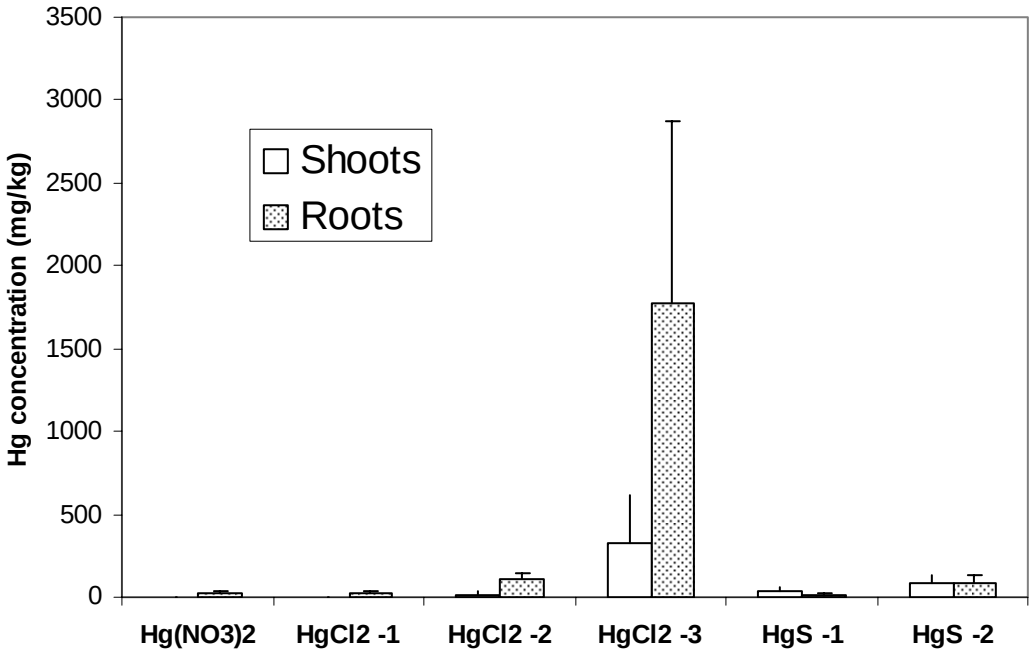

Figure 4. Plant shoot and toot mercury accumulations (in mg/kg dry weight) from aged soil study with different mercury contaminant sources.

SUMMARY

We conducted three sets of experiments to evaluate the phytoremediation potential of three plant species, Indian mustard (*Brassica juncea*), beard grass (*Polypogon monospeliensis*), and Chinese brake fern (*Pteris vittata)*. These include a pot study with potting mix and mercury provided as $HgCl_2$ solution; a second set of experiments with mercury chloride-spiked soil; and a third experiment with Indian mustard in aged soil contaminated by different mercury sources. At the end of the potting mix experiment, both Indian mustard and beard grass plants showed severe stress symptoms resulting from mercury exposure. The Indian mustard plants had to be harvested earlier because of severe chlorosis symptoms. Chinese brake fern accumulated the highest amount of mercury without visual stress symptoms. During the second set of experiments,

mercury was introduced into soil as $HgCl_2$ powder. Beard grass and brake fern plants were transferred into the spiked soil. Beard grass accumulated a very limited amount of mercury in the shoots (<65 mg/kg), even though root mercury accumulation is significant (maximum 2298 mg/kg). Consequently, we conclude that beard grass is not suitable for mercury phytoremediation. However, brake fern accumulated 540 mg/kg and 1469 mg/kg in shoots after 18 days of growing in soils treated with 500 ppm and 1000 ppm $HgCl_2$ powder, respectively. Our results indicate that Chinese brake fern is a potential candidate for mercury phytoextraction. During the third set of experiments with aged soil, the bioavailability and phytoremediation of different forms of mercury contaminants were studied. The mercury contaminant from aged HgS-contaminated soil was still biologically available; and the uptake and accumulated of mercury in roots and shoots of Indian mustard plants reached 79 and 87 mg/kg dry weight, respectively. We are currently conducting systematic experiments to study the mechanism.

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