Bull. Environ. Contam. Toxicol. (1997) 59:763-769


Bulletin of Environmental Contamination and Toxicology

# Variation and Range of Mercury Uptake into Plants at a Mercury-Contaminated Abandoned Mine Site

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Received: 1 April 1997/Accepted: 29 July 1997

Vegetative uptake of heavy metals in plants growing in contaminated areas has received attention since the 1960's for a variety of reasons. The accumulation of heavy metals by agricultural crops is a public health concern. Vegetative sampling has been used to biomonitor soil levels of heavy metals from natural and anthropogenic sources (Rasmussen 1994). Studies of uptake of heavy metals by plants from mining areas has led to some understanding of heavy metal tolerance in plants and mechanisms; by which tolerance occurs (Antonovics et al. 1971, and Baker and Brook 1989).

Mercury loss associated with the amalgamation process of gold recovery from stampmill mining in Idaho has produced occasional high levels of mercury contamination. Mercury and arsenic as high as 200 ppm and 2000 ppm respectively were measured in an abandoned stampmill site near Atlanta, ID (elevation 5,500 ft, montane zone).

This study is a survey of root, stem, and leaf accumulations of Hg in several shrubs and grasses growing at the abandoned stampmill site near Atlanta. An attempt was made to correlate plant uptake with Hg levels in the soil. Also the question was examined, "do different plants show distinctive differences of uptake levels and patterns of Hg distribution?"

## MATERIALS AND METHODS

The study site was bordered on the north and west sides by the Middle Fork of the Boise River. A road formed the east border. The south end of the study site was covered with thick brush and a steep rise just beyond a small creek that ran into the Middle Fork. A grid was formed by running north-south transect lines the length of the area and east-west transect lines the width of the area. This formed 1.55 quadrates, measuring about 13.1 meters on a side.

Soil samples were collected at a depth of 15-20 cm below the surface from the approximate center of each quadrate. If a quadrate had high levels of Hg (>5 ppm) additional samples were obtained from that quadrate. All soil samples were stored

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in 500 mL plastic bags and frozen at -20 °C until analyzed. Prior to analysis, the soil was thawed and sieved through a Fisher brand US Standard brass #10 soil sieve.

Soil samples ranging from 0.1 g to 1.0 g were extracted using a digestion mixture of $HNO_3$ and $H_2SO_4$ (Stewart and Bettany 1982). The extracts were analyzed using a mercury cold vapor method (Standard Method 245. 1, EPA-625/6-74-003a, 1976) with a Thermo Jarell Ashe Atomic Absorbance Spectrometer. The soil samples were analyzed for total Hg only.

Vegetative samples were collected at the same time as the soil samples. The specimens were washed in deionized water. Special attention was given to the roots, which were scrubbed free of soil and rinsed thoroughly. The plants were cut into sections of roots, stems, and leaves, which were wrapped in plastic wrap and frozen at -80 °C until analyzed. About 300 plants were analyzed for total Hg only.

One gram of sample was placed in a bottle and then microwaved at high-power for 12 minutes. Ten mL of 6 N $HNO_3$ were then added and the bottles sealed and stored for 12 hours. They were then autoclaved at 121 °C and 6.2 kg of pressure for 30 minutes, then analyzed for total Hg by the mercury cold vapor method. Two to six plant samples were analyzed from each soil area containing a given Hg level (an assigned threshold level) (Table 1). At least 2 blanks and 4 standards were analyzed for every 28 samples analyzed. Percent of mercury recovered after spiking vegetative samples prior to digestion averaged 89% ($Hg^{2+}$ in solution was top dressed on vegetative surface). Dry weight was determined from a subset of sample placed in an aluminum pan and dried for 24 hours at 105 °C in a laboratory oven.

A one meter square quadrat was used to sample plant frequency. The quadrat frame was placed systematically along each transect line at two meter intervals on both sides of the transect line at each point. Each time a species appeared in a quadrat it was given a count of one. Data collected in this fashion represented about 6% of the area.

## RESULTS AND DISCUSSION

This study showed that the relationship between soil and plant tissue levels of Hg is complex. Two shrubs showed a relatively high significant correlation of root and leaf Hg accumulation with adjacent soil Hg-concentration. Root and leaf Hg to soil Hg correlations were 0.9929 and 0.8682 for *Lonicera involucrata* (Twinberry) and 0.9732 and 0.9287 for *Spiraea douglasii* (Table 1). *Ribes cereum* (Squaw currant), *Amelanchier alnifolia* (Western serviceberry), and *Salix* (Willow) showed root and leaf to soil Hg correlation values of 0.8567, 0.5696, 0.2679. 0.0019, 0.0711, and 0.1787 respectively. One grass, *Dactylis glomerata* (Orchard grass) showed root and stem Hg to soil correlations of 0.9732 and

**Table 1**. Hg in roots, stems, and leaves (ppm dry weight) correlated with soil Hg levels (ppm dry weight)[1].

| Species | Soil Hg Range ppm | Root Hg Range ppm | Stem Hg Range ppm | Leaf Hg Range ppm | # of samples analyzed (plant tissue) |
|---|---|---|---|---|---|
| *Agrpyron sp* | 0–130 | 0.025–17.1 | 0.000–3.61 | | 38 |
| correlation | | 0.9109 | 0.2299 | | |
| *Poa pratensis* | 0–90 | 0.000–43.4 | 0.000–3.02 | | 38 |
| correlation | | 0.1928 | 0.0313 | | |
| Agrostis alba | 0–55 | 0.023–57.6 | 0.000–6.06 | | 48 |
| correlation | | 0.9158 | 0.4238 | | |
| *Dactylis glomerata* | 0–130 | 0.021–67.9 | 0.000–7.52 | | 18 |
| correlation | | 0.9732 | 0.9802 | | |
| *Lonicera involucrata* | 0–130 | 0.000–11.8 | 0.000–0.543 | 0.000–1.80 | 18 |
| correlation | | 0.9929 | 0.1026 | 0.8682 | |
| *Ribes cereum* | 0–129 | 0.000–24.6 | 0.000–6.42 | 0.000–3.65 | 18 |
| correlation | | 0.8567 | 0.0767 | 0.5696 | |
| *Spiraea douglasii* | 0–130 | 0.000-37.4 | 0.000–2.21 | 0.000–8.96 | 44 |
| correlation | | 0.9732 | 0.9170 | 0.9287 | |
| *Amelanchier alnifolia* | 0–130 | 0.000–18.7 | 0.000–3.37 | 0.000–5.37 | 22 |
| correlation | | 0.2679 | 0.0393 | 0.0019 | |
| *Salix sp* | 0-130 | 0.000–16.7 | 0.000–0.345 | 0.000–3.52 | 22 |
| correlation | | 0.0711 | 0.0790 | 0.1787 | |

[1]Linear correlation coefficient values are included. N ranged from 2-4 for each soil and plant sample analyzed.

0.9802 respectively (Table 1) *Agrostis alba* (Redtop bentgrass) and *Agopyron spicatum* (Wheatgrass) showed correlation ($r^2$) values of 0.9158 and 0.9109 for root to soil Hg levels respectively. The stem Hg levels showed much less correlation with soil Hg levels with $r^2$ values of 0.4238 and 0.2299 respectively. *Poa pratensis* (Kentucky bluegrass) showed no correlation of tissue to soil Hg levels with $r^2$ values of 0.1928 for root and 0.0313 for stem tissue.

Cocking et al. (1995) found that tissue Hg concentration was directly related to soil Hg and that regression of the concentration of Hg in individual plants on the Hg content of adjacent soil produced significant correlation in *Asclepias* and *Solidago*. They also found root concentration of Hg was inversely related to the subterranean organ size. Barghigiani and Bauleo (1992) found the mercury levels in *Abies alba* needles correlated with soil Hg levels.

Cocking et al. (1995) make the statement that it is difficult to predict Hg levels in vegetation of Hg-contaminated terrestrial ecosystems due to the large number of variables. One of the major variations is probably mercury speciation differences. As the following discussion suggests, there are many variables that may affect the levels of Hg accumulated in the plants. Since some of the mercury was introduced into the site as elemental Hg more than 100 years ago, multiple Hg species probably are now present. Some elemental Hg remains since some soil samples showed detectable $Hg^{O}$ vapor prior to the $Hg^{2+}$ reduction step to $Hg^{O}$ in the mercury cold vapor method of analysis. In addition, $Hg^{2+}$ from chemical and biological oxidation of $Hg^{O}$, and methyl mercury from microbial methylation are probably present. Laboratory cultures of *Clostridium cochlearium* and *Pseudomonas* sp. have been shown to methylate mercury. *Clostridium* and *Psuedomonas* genera are present in terrestrial soils and may exhibit strains capable of methylating mercury (Trevors 1986). Vascular plants have been shown to differ in mercury uptake depending on the species of mercury presented to the plants. Inorganic mercury and methyl mercury have been shown to be taken up by roots and translocated to needles of *Picea abies* seedlings (Godbold 1994) with a greater accumulation of mercury from inorganic mercury. A greater amount of Hg accumulated in cabbage (*Brassica oleracea*) following soil treatment with methyl mercury compared to soil treatment with mercuric chloride (Bache et. al. 1970). Compounding the problem of understanding the extent of these processes is the possibility that vascular plants may release mercury (Siegel et al. 1974). Also these plants may take mercury up from Hg vapor, a process in which catalase appears to play an important role (Du and Fang 1983). The differences in species abundance of Hg in addition to differences in other soil quality properties such as moisture, pH, soil type and organic matter all play a role in creating differences in Hg accumulation in plants.

Landers et. al. (1995) found that even vegetation from pristine areas in the Arctic contained Hg, but generally in low concentrations. Mosses and lichens containing from 0.04-0.06 ppm Hg were representative in this study. Vegetation growing in Hg-contaminated soils results in plant tissue accumulations of Hg which are 100 fold greater than that contained in pristine vegetation.

In our studies, all 5 shrubs showed leaf values of at least 1.5 ppm Hg with 2 of the shrubs containing more than 5 ppm Hg in the leaves. The Hg values in the grass stems ranged from 3 to 8 ppm Hg for the maximum values. The values are within the ranges others have observed in several trees and a grass: 5.23 ppm in *Abies*

**Table 2.** Plant frequencies associated with soils containing various Hg levels (ppm dry weight)[2].

| Species | Soil Hg Range ppm | Plant Frequency | Relative Plant Frequency |
|---|---|---|---|
| *Spiraea douglasii* | 0.20–1.00 | 27.0 | |
| | 1.10–5.00 | 4.8 | 18% |
| | 5.10–49.9 | 5.2 | 19% |
| | 50.0–130 | 4.9 | 18% |
| *Amelanchier alnifolia* | 0.20–1.00 | 7.5 | |
| | 1.10–5.00 | 2.5 | 33% |
| | 5.10–49.9 | 5.9 | 79% |
| | 50.0–130 | 5.8 | 77% |
| *Lonicera involucrata* | 0.20–1.00 | 4.5 | |
| | 1.10–5.00 | 2.0 | 50% |
| | 5.10–49.9 | 0.1 | 2% |
| | 50.0–130 | 0.2 | 4% |
| *Poa pratensis* | 0.20–1.00 | 17.0 | |
| | 1.10–5.00 | 4.8 | 28% |
| | 5.10–49.9 | 4.0 | 24% |
| | 50.0–130 | 3.5 | 21% |
| *Dactylis glomerata* | 0.20–1.00 | 8.0 | |
| | 1.10–5.00 | 4.9 | 61% |
| | 5.10–49.9 | 4.0 | 50% |
| | 50.0–130 | 3.0 | 38% |

[2]Plant frequency is the average number of plants found in a square meter along selected transects. Relative plant frequency is the plant frequency in the higher soil Hg ranges relative to the plant frequency in the 0.20-1.00 ppm soil Hg range expressed as a percentage.

needles from trees in 85 ppm Hg soil (Barghigiani 1992), 1.5 ppm in pine needles (*Pinus nigra*) and 1.04 ppm in spruce needles (*Abies alba*) from trees in 85 ppm Hg soil (Barghigiani 1994) and 4.83 ppm in shoots from *Cynodon dactylon* in 22

ppm Hg soil (Lenka et. al. 1992). It is not clear whether the values in our study would present a significant toxic risk to wildlife. Concentrations of Hg in herbivores are typically low, however some mammalian herbivores (rabbits and male roe deer) show higher tissue Hg levels in animals sampled in Hg contaminated areas compared to those from non-contaminated areas. Studies show that within the terrestrial food chains, Hg levels are biomangnified as follows: carnivores > omnivores > herbivores (Wren, 1984).

Within the 5 shrubs and 4 grasses that were surveyed, no consistent distinctive differences of root, stem, and/or leaf Hg accumulation patterns were observed. In all plants examined, accumulation of Hg in the roots exceeded that accumulated in stems or leaves. Kahle H (1993) and Punz WF and Sieghardt H (1993) discuss the fact that heavy metals tend to inhibit the growth and function of roots in trees and herbaceous shrubs. This could decrease uptake and translocation of Hg. Root material may bind Hg (e.g. to structural components) thus yielding high root levels of Hg with lower upper plant Hg levels.

Mercury did influence the frequency of plants growing at the site. Table 2 shows plant frequency as a function of soil Hg level. The table shows that the frequency of each plant was greatest in the lesser contaminated soil, which contained 0.2-0.99 ppm Hg and from 80% less for *Spiraea douglasii* and *Poa pratensis* to 14% less for *Amelanchier alnifolia* in the remaining areas of higher contamination levels of Hg.

In conclusion, our studies demonstrate that there is appreciable Hg accumulation (1.5-8 ppm Hg) in plants from soils containing 5 ppm Hg and greater. Mercury levels in the plants from soils of 0 to 130 ppm Hg correlated with the soil levels of Hg in some plants and not others. Plant frequency was reduced in the soils containing higher levels of Hg.

Further studies will look into the nature of the uptake of Hg, e.g. effect of Hg speciation on uptake into plants and the nature of the distribution of Hg within the plant tissue, e.g. Hg-protein binding and Hg binding to other molecules.

Acknowledgments. We thank Wayne Owen for his advice during the preparation of this manuscript. This work was supported in part by the U.S. National Forest Service, Boise National Forest.

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