

## Cadmium, Copper, and Lead in Two Species of *Artemisia* (Compositae) in Southern Manitoba, Canada

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Heavy metal contamination in native plants that are used for herbal remedies and pharmaceutical sources is now recognized as a significant potential source of human health risk (e.g. Olujohungbe et al. 1994, Chan and Critchley 1996, Drew and Myers 1997, Bateman et al. 1998). Compared with metal levels that are found in most food products, dried herbs may contain comparatively high concentrations (WPMF 1973, Keen et al. 1994).

The genus *Artemisia* (Compositae) has long been used in the traditional folk medicines of many cultures, and is currently under examination for potential therapeutic agents in the treatment of inflammatory fungal, bacterial and viral infections, malaria, hepatitis, and cancer (Tan et al. 1998). In Manitoba, *Artemisia absinthium*, a naturalized species, and *A. ludoviciana*, native to North America, are often harvested for folk and naturopathic remedies. *Artemisia absinthium* has been investigated as an anthelmintic and antiparasitic (Guarrera, 1999), antipyretic (Khattak et al., 1985), and hepatoprotective (Gilani and Janbaz 1995) agent, as well as for memory improvement (Wake 2000), and a number of other applications (Maw et al. 1985, Gilani and Janbaz, 1995). Extracts of *A. ludoviciana* have shown antimalarial properties (e.g. Malagon et al. 1997).

Little information exists on the metal-accumulating properties of these perennial herbs, even though they are commonly found in disturbed areas with a greater likelihood of metal contamination. Chizzola and Franz (1996) analyzed nine samples of commercially grown *A. absinthium* from Austria and identified some samples with high values for cadmium and lead. Nwosu et al. (1995) found that interspecific differences existed among crop plants in the uptake of Pb and Cd, and suggested that a large array of plant and soil factors (for example pH, organic matter content, particle size, oxides of manganese and iron) may influence uptake rates. Speciation of individual metals in various soil types (Krishnamurti et al. 1997) and differential mobility of different trace metals (Marr et al. 1997) may also affect their availability for uptake.

The objective of the present study was to examine Cd, Pb and Cu levels in roots, leaves and inflorescences of *A. absinthium* and *A. ludoviciana*, and the corresponding soils, from a number of sites in southern Manitoba.

MATERIALS AND METHODS

Whole, flowering plants that were growing wild were collected during 11-17 August, 1999 at 34 sites in southern Manitoba within the area bounded by 49-51° N and 95-99° W. The sites included agricultural fields, areas adjacent to roadways and railway tracks, and vacant lots. Soil types varied from clay to gravel, with a wide range of moisture conditions. The two species of *Artemisia* did not occur together at the same sites.

Soil was washed from roots, the plants were air-dried, and stored at 4°C. The top 5 cm of soil were sampled using a wooden spatula and air dried. Roots, leaves and inflorescences were ground to a powder in a small mill. Samples of 0.1 g were digested for 30 minutes in 1:1 v/v concentrated nitric acid and 30% hydrogen peroxide in closed acid-washed test tubes in a water bath at 60°C. At least three 100 µL aliquots of each digestion liquor were analyzed using a PDV 2000 digital anodic stripping voltammeter (Chemtronics Ltd., Bentley, Australia) to obtain mean values for Cd, Cu and Pb. The standard additions method (Mann et al. 1974) was used to compensate for matrix absorption effects, using incremental additions of metals as certified atomic absorption standards (Fisher Scientific Co., Fair Lawn, New Jersey). Blanks consisted of all reagents and procedures less sample material. Recovery estimates using samples spiked prior to digestion yielded rates of 90-95%.

Soil organic matter content was determined by igniting 2 g of soil in a muffle furnace at 500°C for a total of 2 hours. The critical significance level for all statistical tests was  $p = 0.05$ .

RESULTS AND DISCUSSION

Metal concentrations in the soils (Table 1) showed a wide range of values The highest levels of Pb were found at sites adjacent to highways as a result of historic deposition from leaded fuels. Cu concentrations were generally low. Cd and Cu showed a strong exponential positive correlation with each other in the soils ( $r=0.51$ ,  $p=0.001$ , both parameters log transformed). Both Cd and Cu (each log transformed) were significantly positively correlated with soil organic matter content ( $r=0.34$ ,  $p=0.03$  for both metals).

Table 1. Metal concentrations (µg/g dry weight) in soils at the collection sites.

	<i>A. absinthium</i> (N= 19)		<i>A. ludoviciana</i> (N= 14)	
	Mean (± S.E.)	Range	Mean(± S.E.)	Range
Cadmium	1.16 (0.64)	<0.1-11.6	0.75 (0.47)	<0.1-6.05
Lead	29.0 (7.8)	<0.1- 110	11.5 (3.5)	<0.1- 52
Copper	1.03 (0.48)	<0.1- 8.1	1.68 (1.04)	<0.1-14.2

Metal concentrations in the plant organs (Table 2) showed higher mean values than those reported for Austrian herbal *A. absinthium* samples (Chizzola and Franz 1996) due to the more contaminated nature of many of the roadsides where the plants were found in the present study. Toxicity to plants has been reported at soil Pb levels as low as 100 ug/g dry weight (Kabata-Pendias and Pendias 1985); few soils in the present study exceeded this value. Accordingly, the majority of plant Pb levels in the present study corresponded with the range of 0.1-10 ug/g dry tissue weight reported for plants considered to be growing in relatively uncontaminated soils (Kabata-Pendias and Pendias 1985).

**Table 2.** Metal concentrations (ug/g dry weight) in the organs of the two species.

<i>A. absinthium</i>	Roots		Flowers		Leaves	
	Mean (± S.E.)	Range	Mean (± S.E.)	Range	Mean (± S.E.)	Range
Cadmium	0.22 (0.06)	<0.1-1.00	0.76 (0.17)	0.06-3.10	0.42 (0.09)	0.07-1.63
Lead	9.35 (2.93)	<0.1-44.0	11.2 (2.65)	<0.1-45.6	9.78 (2.54)	<0.1-39.3
Copper	14.3 (3.80)	<0.1-48.9	23.3 (4.95)	<0.1-69.4	21.6 (4.51)	<0.1-64.0
<i>A. ludoviciana</i>	Roots		Flowers		Leaves	
	Mean (± S.E.)	Range	Mean (± S.E.)	Range	Mean (± S.E.)	Range
Cadmium	0.14 (0.03)	0.04-0.33	0.83 (0.16)	0.09-2.17	0.48 (0.15)	<0.1-1.61
Lead	9.95 (0.46)	<0.1-61.5	9.92 (3.23)	<0.1-42.5	4.07 (2.00)	<0.1-21.5
Copper	12.6 (4.39)	<0.1-49.6	24.7 (8.7)	<0.1-108.3	18.5 (5.18)	<0.1-66.9

Pb concentrations in pooled organ samples showed strong linear correlations with Pb levels in soil for both *A. absinthium* ( $r=0.38$ ,  $p=0.002$ ) and *A. ludoviciana* ( $r=0.50$ ,  $p<0.001$ ). Plant and soil Pb levels in *A. absinthium* were also significantly correlated for each of the roots and flowers, but less so for leaves. In *A. ludoviciana* the plant-soil relationship was highly significant for roots ( $r=0.87$ ,  $p<0.001$ ), and still positive but not significant in leaves and flowers.

Tissue metal concentrations were not significantly different between the two species, either for whole plants or for respective organs. Cd and Cu in tissues were less correlated with soil concentrations than was the case for Pb, perhaps because of the lower ranges of values for Cd and Cu in soils. For Cd, soils at the majority of the sites were within the range of 0.01 - 7 ug/g considered unpolluted according to Allaway (1968). While inflorescences tended to show the highest mean values for the three metals in both species (Table 2), these differences were not significant because of the high degree of overlap at the lower concentration ranges.

In *A. absinthium*, Cd concentrations were significantly correlated between flowers and leaves ( $r=0.47$ ,  $p=0.02$ ), while Pb was correlated between roots and leaves ( $r=0.46$ ,  $p=0.02$ ) and roots and flowers ( $r=0.69$ ,  $p<0.001$ ). Cu in this species was also correlated between roots and leaves ( $r=0.58$ ,  $p=0.004$ ), roots and flowers ( $r=0.65$ ,  $p=0.001$ ), and leaves and flowers ( $r=0.67$ ,  $p=0.001$ ). In *A. ludoviciana*, fewer correlations were found among organs: leaves and flowers showed significant

positive correlations for each of Pb ( $r=0.78$ ,  $p<0.001$ ) and Cu ( $r=0.79$ ,  $p<0.001$ ). Roots and flowers were also significantly correlated for Cu ( $r=0.58$ ,  $p=0.015$ ). The latter species showed no significant inter-organ correlations for Cd.

In *A. absinthium*, Pb in whole plants was highly significantly linearly correlated with copper ( $r=0.45$ ,  $p<0.001$ ), while in *A. ludoviciana*, Cu and Pb were exponentially positively related ( $r=0.58$ ,  $p<0.001$ ). The correlation between Pb and Cu remained significant when all three organs of *A. absinthium* were examined separately, but was strongest in the leaves ( $r=0.63$ ,  $p=0.002$ ). For *A. ludoviciana* the exponential relationship between Pb and Cu also persisted in all three organs individually, although in this species the strongest correlation was in roots ( $r=0.79$ ,  $p=0.001$ ).

The tissue/soil ratios for metal concentrations were not significantly different between the two species for any of the metals when all organs were pooled. However tissue/soil ratios were significantly negatively correlated with soil concentrations for Cd ( $r=-0.30$ ,  $p=0.027$ ), Pb ( $r=-0.28$ ,  $p=0.039$ ) and Cu ( $r=-0.26$ ,  $p=0.05$ ) for *A. ludoviciana*, and similarly for Cd ( $r=-0.28$ ,  $p=0.019$ ) and Cu ( $r=0.37$ ,  $p=0.002$ ) for *A. absinthium*. These findings suggested that proportionately less uptake occurred at the higher soil metal concentrations. A similar finding has been reported for these metals in aquatic macrophytes (Pip and Stepaniuk 1992).

Tissue/soil ratios ranged from 2-8 for Cd and Pb. However both species accumulated Cu to a considerable extent. In *A. absinthium*, ratios ranged from a mean of 120 in roots to 200 in inflorescences; similarly for *A. ludoviciana*, these values ranged from 98 in roots to 208 in inflorescences. Leaves showed intermediate values. According to Raskin et al. (1994), Cu/Co accumulators differ from species that are Cd/Pb/Zn accumulators, and from Ni accumulators. The results of the present study conformed with this categorization where Cu was accumulated to high levels, but Cd and Pb were not.

In conclusion, while plant Pb levels showed the greatest tendency to reflect soil concentrations, all three metals were accumulated proportionately less at higher soil metal levels, suggesting that the most efficient uptake occurs at lower soil concentrations. The strong ability of these two species to accumulate Cu should be considered when these plants are exploited for medicinal uses.

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