Preliminary Results of Field Trials Testing Plants for Phytoextraction Capability in a Multi-metal Contaminated Environment Near a Lead Zinc Smelter

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Abstract

Screening of more than 100 members of the *Brassica* family and other species was completed at a University of Guelph greenhouse. Based on an extensive review of published reports plants were selected for future phytoremediation work on a site close to a smelter in Trail affected by particulate and plume deposition from stack emissions. The area chosen for research had been assayed and found to have high concentrations of many metals.

Twelve plants were selected for field trials. Six replicates of five treatments were used: control; addition of biosolid residuals in a 50% mix with existing soil; addition of 25% peat, 25% biosolid residuals and existing soil; and the same two treatments with the addition of *Penicillium bilaii*.
Soil was sampled, and re-sampled after amendment addition. Plants were harvested at 6 & 12 weeks or when dead and assayed for metal content using ICP-MS. At harvest, a soil sample was taken from below the plant and assayed. Final harvest took place in October and plants were dried and weighed to provide an estimate of biomass production.

Results show several plants that have potential for phytoremediation in this area and that metal concentration in the soil for the four major contaminants was reduced from 10 B 25% in a year.

1.0 Introduction

Soil and water contamination can be a concern in areas near mining and smelting. Production processes have changed to reduce and/or eliminate further negative environmental impacts and problems associated with long-term activity are being addressed. The effects of 50-100 years of deposition of fine metal particulate coupled with acidification due to plume effects have resulted in acidic soil that has high levels of heavy metals.

Researchers and scientists have turned their attention to a promising new technologies B rhizofiltration and phytoremediation (Salt et al, 1995, Dushenkov et al, 1995). It is gaining favour amongst industries, regulatory agencies and the general public as it is very low impact and utilizes processes that are easily understood. The process rapidly returns the environment to a normal appearance. Special cultivars able to withstand the toxic effects of metals can be used to stabilize or remove metals. Such plants are found growing in areas that are known to be high in metals or plants are developed (genetically or by hybridization) to maximize their ability to safely sequester metals.

This process is safe, relatively inexpensive compared to other methods and potentially very cost effective. The affected soil may require amendments to support plant growth and careful plant selection is also a prerequisite. Instead of removing soil and replacing with purchased topsoil suitable plants are grown on the site and subsequently harvested. The resultant metal containing biomass can be treated to remove metals (smelted) or disposed of in a much smaller area (say by ashing). However, no site has yet been decommissioned using this technology alone.

The ideal plants for purposes of phytoextraction are hyperaccumulators. For Zn, Pb, Ni, Cu or Mn a plant is described as a hyperaccumulator that can safely store more than 1% dry weight while for Cd the level is 0.01% (Baker et al, 1991). To be most cost effective the plants should store the metal in an easily harvestable part.

To date research has concentrated on removal of one, or at most two elemental metals of concern. However, sites near smelters pose particular problems. Generally, the soil has numerous heavy metal contaminants ranging from levels similar to normal background levels to levels that require remediation to meet Provincial regulations. Metals of primary concern at Trail are Pb, Zn, Cd and As.

2.0 Method

2.1 Greenhouse Research

In fall 1997, soil samples were taken from areas around Trail and shipped to the University of Guelph. Initially, 100 *Brassica* family members were screened using hydroponics systems to assess their abilities to grow in metal-contaminated water. Two areas were then investigated:

1) The potential of seeds of specific species said to be heavy metal resistant to germinate and establish successful seedlings on soil found in the Trail area.
2) The potential of germinated seedlings of the same species successfully being transplanted into soil that was either as found *in situ* or modified using a number of amendments.

2.2 Field Studies

From greenhouse research potential candidates were selected for field trials during the summer of 1998. Plants were grown in a local greenhouse and transplanted in late May. The majority were *Brassicaceae* but other species were investigated: a native grass; *Agrostis stolonifera*; two native trees: horse chestnut, (*Aesculus hippocastanum*) and silver maple, (*Acer saccharinum*); a non-native tree species B hybrid poplar (*Populus deltoides)*; lemon scented geraniums (*Pelargonium* reported by Saxena et al, 1998); and *Eruca sativa* or arugula.

The *Brassica* planted were: kale (*Brassica oleracea*), flowering kale (*Brassica oleracea*), flowering cabbage (*Brassica oleracea*), BCN 3483 (mizuna) (*Brassica rapa, japonica group*), turnip greens (shoigun) (*Brassica napa*), Cime di rapa broccoletti (*Brassica rapa*), wild wallflower (*Cheiranthus cheiri*), radish (Mino summer cross) (*Raphanus spp.*)

There were 5 treatments: Addition of 50% biosolid residuals; Addition of 25% peat & 25% biosolid residuals; Addition of 50% biosolid residuals & *Penicillium bilaii*; Addition of 25% peat, 25% biosolid residuals & *Penicillium bilaii*; and a Control (no amendments). Six replicates were prepared with treatments distributed randomly in each replicate. Each single plot was thoroughly tilled. *Penicillium bilaii* was added per manufacturer=s instructions to investigate its potential as a natural chelator. Nine plants of each species (except for trees) were sown into each plot (330 of each species). Sub-plots were randomly laid out in each of the 30 plots and the area fenced. Soil was assayed in each plot before and after amendments were added. A random sample (top 8 cm) was taken from three places in the plot and mixed together in a plastic labeled bag. A generous amount of calcium nitrate was added to each plot, including controls, after sampling was completed.

Weekly, plots were monitored and plant growth, colouration and physical changes noted. Watering was carried out regularly. A water-soluble fertilizer was twice added according to manufacturer=s instructions. In June and August, a single plant was harvested from each sub-plot. Plants were separated into root and shoot, placed in labeled paper bags, dried at 80□C for 24 hours, digested (Campbell and Plank, 1998) and assayed. At final harvest, a soil sample was collected below the root area where the plant had grown and placed in labeled paper bags, air-dried, digested (Soong, 1998) in Nitric Acid and assayed. One complete plant of each species from each plot was harvested as above. Biomass was determined and used to calculate total plant uptake potentials.

3.0 Results

Soil amendments increased plant growth and biomass while control plots with no amendments had plants that barely survived with some growing slowly but most plants in control plots died over the course of the summer.

Table 1 Final biomass determinations (dry grams per individual) of each species in each treatment. Roots and shoots were assayed separately and metal concentrations combined except for maple and poplar where only leaves were harvested.

Species	Control		Soil, Biosolid 50:50		Soil, Biosolid plus *Penicillium b.*		Soil, Peat & Biosolid		Soil, Peat, Biosolid plus *Penicillium b.*	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E	mean	S.E.
Cabbage	1.48	0.46	26.38**ab**	3.75	31.82**a**	10.06	33.11**a**	7.54	26.5**ab**	6.22
Eruca sativa	0.76	-----	12.35	6.29	9.71	4.16	10.06	3.28	15.28	4.75
Geranium (l)	-----	-----	222.88	38.58	204.6	52.22	177.49	36.16	166.32	43.13
Geranium (s)	3.15**b**	0.86	9.38**ab**	2.49	7.77**ab**	1.55	14.62**a**	4.21	8.40**ab**	2.21
Kale	1.64	0.53	17.58	6.12	17.92	3.07	18.27	4.33	20.41	5.67
Flowering Kale	1.49**b**	0.34	11.53**b**	1.10	19.81**ab**	3.07	38.20**a**	11.12	22.06**ab**	5.56
Maple, silver	0.17	0.05	2.03	0.13	0.37	0.29	1.15	0.73	------	-----
Poplar, hybrid	0.05	-----	3.48	0.88	2.65	0.64	2.34	0.42	2.63	0.64
Radish (Mino)	15.82	1.80	10.45	2.22	32.03	8.44	14.89	4.74	21.10	8.26
Rapa	-----	------	3.77	1.97	5.39	1.63	4.73	1.73	3.33	2.52
Redtop	15.82	1.80	15.97	3.78	22.36	5.71	13.80	3.41	15.61	8.25
Shoigun	6.36	-----	7.76	1.41	9.65	2.82	5.80	3.36	6.64	1.75
Wallflower	0.78	0.04	3.58	1.30	7.08	1.96	4.24	1.55	2.60	0.95

F ratio for the specified treatment is significant at the 95% confidence level if Prob>F is less than or equal to 0.05. a,b,c, means any given species and plant part sharing the same letter are not significantly different at the p=0.05 level as determined using the Tukey multiple comparison test.

Treatment with 25% biosolid residuals and 25% peat was best for growth of flowering cabbage, small geraniums and flowering kale (Table 1). The treatment with only biosolid residuals produced the highest biomass for wild wallflower. All other species showed no significant biomass differences among treatments.

Table 2 Elemental concentrations in all species under different amelioration treatments; root shoot combined.

Treatment	Number of Samples	As, µg/g mean	As, µg/g S.E.	Cd, µg/g mean	Cd, µg/g S.E.	Pb, µg/g mean	Pb, µg/g S.E.	Zn, µg/g mean	Zn, µg/g S.E.
Garden	12	6.51	1.42	9.26	1.48	97.93	22.12	663.26	100.81
Control	51	16.48	1.76	33.61	4.50	407.09	59.52	2511.26	233.31
Soil, Biosolids 50:50	184	9.39	0.90	24.83	1.14	179.61	21.72	1265.42	45.68
Soil, Biosolids plus *P.b.*	175	7.82	0.54	24.79	1.77	121.24	10.52	1190.35	72.67
Soil, Peat & Biosolids	181	11.86	1.19	26.49	1.23	217.78	25.24	1651.89	60.60
Soil, Peat, Biosolids, *P.b.*	166	8.80	0.98	25.27	1.21	166.87	20.65	1603.96	71.10
Anova Results									
Random Block Design									
Treatment Effects	F	3.36		0.95		4.58		8.72	
	Prob>F*	0.0293		0.4563		0.0087		0.0003	

Notes: divide concentration in µg/g by the element's atomic weight to give its molecular concentration in µmol/g analysis of variance results remain identical except for combinations of several elements.
a,b,c, means for any given element sharing the same letter are not significantly different at the p=0.05 level as determined using the Tukey multiple comparison test.
*F ratio for the specified treatment effect is significant at the 95% confidence level of Prob>F is less than or equal to 0.05

The effect of each soil amendment treatment on the total metal accumulated across all species was calculated with individual assay results for As, Cd, Pb and Cd given (Table 2). Total accumulation in all species for these four metals was also calculated (Table 3).

There were some differences in mean plant uptake due to treatment effects:

- For As the 50:50 biosolid treatment had a significantly lower plant uptake than in control plants, as did the treatment that utilized peat diluted biosolid residuals and *Penicillium bilaii.*
- There were no differences for Cd in plant uptake between controls and any treatment
- The highest plant uptake of Pb was in the control and the total was significantly different than for plants grown in any of the treatments. However, there were no significant differences in plant Pb uptake between any of the treatments.
- Plant uptake of Zn was highest in control plots and this was statistically significantly different than for any of the treatments. There were differences between some of the treatments with the lowest level obtained with the 50:50 biosolids treatment plus the addition of *Penicillium bilaii*.
- *Penicillium bilaii* did not appear to increase metal uptake, as there were no significant differences

.Table 3 Concentrations of four elements (As, Cd, Pb, Zn) in all species under different amelioration treatments; root and shoot combined.

Treatment	Number of Samples	As, Cd, Pb, Zn µg/g mean	S.E.	As, Cd, Pb, Zn µmol/g mean	S.E.
Garden	12	776.97	109.64	10.79	1.59
Control	51	2968.44	237.54	40.90	3.58
Soil & Biosolid (50:50)	184	1479.24	56.76	20.57	0.75
Soil, Biosolid, & *P.b.*	175	1344.20	76.39	19.12	1.15
Soil, Peat & Biosolid	181	1908.30	71.81	26.71	0.98
Soil, Peat, Biosolid, *P.b.*	166	1804.90	80.76	25.68	1.13

Anova Results			
Random Block Design			
Treatment Effects	F	8.87	8.82
Prob>F*	0.0003	0.0003	

Mizuna (*Brassica rapa, japonica* group) sequestered the highest concentrations of As, and Pb (Table 4). *Brassica oleracea,* (Cime di rapa broccoletti) sequestered the highest concentrations of Cd and Zn. The highest total metal concentration was found in Cime di rapa broccoletti *(Brassica rapa)* and *Eruca sativa* (Table 5). Shoigun (*Brassica napa*) and mizuna followed with no differences between these two and *Eruca sativa* at less than 0.3% in terms of dry weight. While *Eruca sativa* had low variability from plant to plant, other plants displayed large variability in the total metals sequestered.

Plant uptake by all species varied significantly with treatment (Table 6). Soil amended with biosolid residual diluted with peat supported the highest plant uptake of As, Pb, and Zn and the sum of the four metals - µg or µmol (Table 7) . Highest loading of Cd was found in 50:50 biosolids with *Penicillium bilaii* treatment.

Total element loading among species across all treatments varied with plant biomass. The large biomass produced by the geraniums resulted in relatively high metal loading in all treatments (data not shown). The greater biomass of geraniums means that three times the total metals were removed than by Mizuna (ranked second). This is an important consideration in determining plants for future research.

Total elemental loading: metals accumulated in root or shoot (or both) are multiplied by the biomass of the respective plant part resulting in values for an entire plant (Table 8). The calculation factors in biomass as the larger a plant grows the higher the total metals taken up are for the plant. The metal value data is taken from the second harvest. Values are given for whole plants.

Table 4 Elemental concentrations in different species across all amelioration treatments; root and shoot combined. Mean levels of metal concentration as determined by ICP - AES analysis were computed for As, Cd, Pb and Cd.

Species	Number	As,µg/g Mean	S.E.	Cd,µg/g Mean	S.E.	Pb,µg/g Mean	S.E.	Zn,µg/g Mean	S.E.
Cabbage	51	3.73 **de**	0.63	27.67 **cde**	4.79	76.84 **cde**	13.70	1551.20**cde**	194.69
Eruca sativa	52	14.16**ab**	1.40	38.77 **ab**	2.94	261.96 **a**	37.38	2140.79 **ab**	120.00
Geranium	123	10.44 **bc**	0.89	17.73 **ef**	1.45	222.48 **abc**	29.05	1041.54 **fg**	82.78
Kale	50	4.24 **de**	0.50	26.24 **cde**	1.49	104.23 **bcd**	15.09	1405.53 **def**	62.31
Kale (flowering)	49	2.55 **e**	0.39	19.45 **de**	0.93	54.43 d	9.91	1135.34 **efg**	46.10
Mizuna	99	19.41**a**	2.23	29.06 **bcd**	1.82	282.79 **a**	38.89	1653.61 **cd**	86.38
Hybrid Poplar	22	3.06 **de**	0.34	30.93 **bc**	3.29	48.14 d	8.81	1319.17 **def**	86.67
Radish (Mino)	103	8.69 **bcd**	0.84	19.83 **de**	1.34	182.59 **abcd**	27.25	1241.66 **defg**	85.29
Rapa	46	10.12 **bc**	1.1	46.28 **a**	3.41	251.50 **ab**	35.53	2494.73 **a**	142.95
Redtop	24	5.2 **cde**	0.6	8.45 **f**	1.55	100.24 **bcd**	19.67	837.19 **g**	70.23
Shoigun	96	13.57 **b**	1.52	29.10 **bcd**	1.82	221.12 **abc**	39.90	1910.33 **bc**	112.07
Wallflower	51	5.60 **cde**	0.94	21.61 **cde**	1.21	140.48 **abcd**	32.04	1104.38 **efg**	69.69
Anova Results									
Random Block Design									
Treatment Effects	F	13.75		18.24		5.28		30.65	
Prob>F	0.0001	0.0001		0.0001		0.0001			

Notes: divide concentration in µg/g by the element's atomic weight to give its molecular concentration in µmol/g

analysis of variance results remain identical except for combinations of several elements.

a,b,c, means for any given element sharing the same letter are not significantly different at the p=0.05 level as determined using the Tukey multiple comparison test.

*F ratio for the specified treatment effect is significant at the 95% confidence level of Prob>F is less than or equal to 0.05

Table 5 Concentrations of four elements (As, Cd, Pb, Zn) in all species under different amelioration treatments; root and shoot combined.

Species	Number of Samples	As, Cd, Pb, Zn µg/g Mean	S.E.	As, Cd, Pb, Zn µmol/g Mean	S.E.
Cabbage	51	1659.43 **cde**	298.10	24.40 **cde**	3.07
Eruca sativa	52	2455.67 **ab**	134.85	34.55 **ab**	1.90
Geranium	123	1292.29 **ef**	90.11	17.30 **ef**	1.29
Kale	50	1540.23 **de**	68.75	22.29 **de**	0.98
Kale (flowering)	49	1211.77 **ef**	49.28	17.84 **ef**	0.72
Mizuna	99	1984.87 **bcd**	106.57	27.18 **bcd**	1.41
Hybrid Poplar	22	1401.30 **ef**	88.25	20.73 **def**	1.34
Radish (Mino)	103	1452.77 **def**	99.02	20.17 **def**	1.37
Rapa	46	2802.63 **a**	161.59	39.92 **a**	2.28
Redtop	24	951.08 **f**	82.78	13.44 **f**	1.14
Shoigun	96	2174.12 **bc**	125.13	30.73 **bc**	1.76
Wallflower	51	1271.77 **ef**	96.10	17.84 **ef**	1.19
Anova Results					
Random Block Design					
Treatment Effects	F	26.15		29.31	
	Prob>F	0.0001		0.0001	

Table 6 Total element sequestration in all species under different amelioration treatments; root and shoot combined. Values are based on assays of plants harvested at the end of the summer only.

Treatment	Number of Samples	As, µg/g mean	As S.E.	Cd, µg/g mean	Cd S.E.	Pb, µg/g mean	Pb S.E.	Znµg/g mean	Zn S.E.
Control	9	30.4 **a**	9.7	29.6 **b**	8.2	802.0 **a**	218.0	3515.6 **b**	1045.5
Soil, Biosolids 50:50	60	194.3 **a**	63.3	481.0 **ab**	108.9	5202.6 **a**	1921.1	31805.1 **ab**	8318.4
Soil, Biosolids plus *P.b.*	56	264.2 **a**	74.1	709.2 **a**	132.4	5474.4 **a**	1630.5	42642.5 **ab**	8376.2
Soil, Peat & Biosolids	62	294.7 **a**	101.4	697.6 **a**	215.1	8969.4 **a**	3662.3	52644.9 **a**	14770.7
Soil, Peat, Biosolids, *P.b.*	61	167.0 **a**	50.3	460.8 **ab**	100.9	4172.8 **a**	1424..3	37178.9 **ab**	8391..3
Anova Results									
Random Block Design									
Treatment Effects	F	6.71		4.01		4.38		3.93	
	Prob>F*	0.0014		0.0150		0.0105		0.0163	

Notes: divide concentration in µg/g by the element's atomic weight to give its molecular concentration in µmol/g analysis of variance results remain identical except for combinations of several elements.
a,b,c, means for any given element sharing the same letter are not significantly different at the p=0.05 level as determined using the Tukey multiple comparison test.
*F ratio for the specified treatment effect is significant at the 95% confidence level of Prob>F is less than or equal to 0.05

Table 7 Combined total element sequestration of As, Cd, Pb and Zn in all species under different amelioration treatments; root and shoot combined. Values are based on assays of plants harvested at the end of the summer only.

Treatment	Number of Samples	As, Cd, Pb, Zn µg/g mean	As, Cd, Pb, Zn µg/g S.E.	As, Cd, Pb, Zn µmol/g mean	As, Cd, Pb, Zn µmol/g S.E.
Control	9	4377.6	1266.9	58.3	17.2
Soil & Biosolid (50:50)	60	37693.0	10076.5	518.7	136.4
Soil, Biosolid, & *P.b.*	56	49090.0	10119.9	688.6	137.6
Soil, Peat & Biosolid	62	62606.6	17993.7	858.8	242.6
Soil, Peat, Biosolid, *P.b.*	61	41979.5	9900.9	595.2	136.4
Anova Results					
Random Block Design					
Treatment Effects	F	4.86		4.29	
	Prob>F*	0.0066		0.0115	

While the effectiveness of specific plants and their abilities to remove and sequester metals from the metal contaminated soil can be seen, soil response is not as clear due to the extreme heterogeneity of soil. Sampling from different plots shows wide variability and even in each plot the sub-samples following amendments exhibit much the same trait.

Figure 1 Graphs of metal reduction of elements (Cd and As; Pb and Zn) during 8-week growth period during summer 1998. Initial sample was taken prior to amelioration additions and prior to all planting. Sample 2 was taken mid summer, sample 3 was taken at the end of the summer following all harvesting.

The data in Table 8 clearly shows the accumulation ability of the tested species. As samples of soil prior to planting were assayed, then re-assayed immediately following the growing season reduction in soil metal presence could easily be determined. It can be seen that all metals of interest were reduced in the soil over the course of the summer (Figure 1). However, it is not clear from the assays whether or not the metals were taken up by plants or were simply removed as they were dissolved in either rain or irrigation water. Table 8 provides a partial answer to this question. It is derived by calculation using the presence of the metal in the soil as assayed prior to planting and presenting this as a ratio to the presence of the metal in plant tissue parts when these were assayed at the end of the summer. If the value of metal concentration is greater than 1, then it indicates that there is an accumulation or concentration of the metal in question in the plant tissue.

The data clearly shows that none of the plants we used were concentrating Pb in their tissues. However, this cannot rule out the potential that some of these might be better accumulators than shown as the Pb in the soil in the area is not present in a soluble form and therefore it is not bioavailable.

For Zn and Cd there are many plants with high accumulation rations. Since both metals are much more soluble in the soil conditions under study, this is expected. Zn, a metal that is a required trace element for plant growth can accumulate to high levels in plants without plant phytotoxicity, whereas, Cd, with no known biological function in plants often leads to phytotoxic plant reactions. Some plants are able to withstand high levels of Cd due to the formation of phytochelatins which effectively bind the metal thereby removing it from plant vital functions.

Eruca sativa, demonstrated an ability to concentrate As in its tissues to a level that is higher than that found in background soil levels. The relatively low value combined with the low general level in terms of As concentration in plant tissue (Table 4) indicate that although it shows an ability to concentrate the metal it is not a hyperaccumulator.

The reduction of metal concentration in the soil over the duration of the experimental process shown as the mean concentration of all metals in the research plot decreases over the course of the first two harvests (Figure 1). Decline over initial values of 23.3% for As, 22.9% for Cd, 10.9% for Pb and 21.5% for Zn occurred during the study period. Metal levels are reduced in soils with no significant differences between treatments.

Table 8 Accumulation of metals in plant tissue; values are mean ratios of plant concentration to soil concentration. Species are listed in order; in descending order of the overall greatest to lowest accumulation of metal relative to that found in the associated soil. Letter following plant name identifies plant part R: root; S: shoot; B: both.

Species		Number	As	Cd	Pb	Zn
Rapa	B	26	0.290 **a**	5.802 **ab**	0.414 **abc**	4.362 **b**
Eruca sativa	S	1	0.261 **ab**	7.422 **a**	0.135 **a**	6.066 **a**
R	1	0.102 **b**	2.908 **ab**	0.091 **a**	2.375 **a**	
B	26	1.576 **a**	5.150 **abc**	0.676 **a**	4.300 **b**	
Mizuna	S	26	0.358 **ab**	4.573 **ab**	0.418 **a**	3.536 **ab**
R	26	0.740 **a**	2.718 **ab**	0.272 **a**	1.927 **ab**	
B	5	0.489 **a**	3.822 **bcd**	0.464 **ab**	2.506 **bc**	
Shoigun	S	26	0.238 **ab**	4.025 **ab**	0.339 **a**	3.630 **ab**
R	26	0.511 **ab**	2.642 **ab**	0.169 **a**	2.439 **a**	
B	6	0.259 **a**	7.570 **a**	0.194 **bc**	6.681 **a**	
Radish Mino	S	25	0.340 **ab**	3.373 **ab**	0.431 **a**	2.706 **b**
R	24	0.242 **ab**	1.465 **ab**	0.144 **a**	1.341 **ab**	
B	7	0.265 **a**	2.636 **cde**	0.265 **bc**	2.658 **bc**	
Kale	S	2	0.553 **a**	2.371 **b**	0.617 **a**	1.711 **b**
B	25	0.117 **a**	2.905 **cde**	0.142 **bc**	2.262 **bc**	
Geranium	S	31	0.317 **ab**	1.010 **b**	0.481 **a**	2.295 **a**
R	32	0.262 **ab**	3.919 **a**	0.353 **a**	2.295 **a**	
B	6	0.231 **a**	1.986 **de**	0.369 **abc**	1.953 **c**	
Maple or	S	23	0.115 **b**	4.606 **ab**	0.110 **a**	2.601 **b**
Poplar	R	1	0.017 **b**	0.191 **b**	0.008 **a**	0.094 **b**
Cabbage	B	27	0.121 **a**	3.764 **bcd**	0.152 **bc**	2.955 **bc**
Wallflower	B	26	0.203 **a**	2.468 **de**	o.249 **bc**	1.719 **c**
Flowering Kale	B	26	0.077 **a**	2.612 **cde**	0.091 **c**	2.121 **c**
Agrostis Stolonifera	B	23	0.170 **a**	1.103 **e**	0.160 **bc**	1.5222 **c**
Anova Results for Shoots						
F			6.13	8.88	4.20	10.52
Prob.>F			0.0009	0.0001	0.0068	0.0001
Anova Results for Roots						
F			21.65	6.41	4.20	10.30
Prob.>F			0.0001	0.0022	0.9576	0.0002
Anova Results for Both						
F			1.03	12.70	10.24	9.09
Prob.>F			0.4360	0.0001	0.0001	0.0001

If Prob.>F is less than 0.05 then treatment effects are significant at the 95% confidence level. Results sharing the same letter (abcde) when in the same row are not significantly different at the 95% confidence level.

By combining information on soil metal reduction with plant uptake insights into the potential of using plants tested to remove metals from highly affected soil can be observed (Figure 1; Table 8). It is possible that treatments that took the form of repeated annual cropping using metal sequestering plants would result in sufficient reductions to meet soil regulatory standards. However, the potential offsite migration of metals dissolved in water needs to be determined to provide a definitive answer. By capturing ground water and assaying it for dissolved and suspended metals it would be possible to complete a thorough mass balance assessment to determine what percentage of the soil metal reduction was due to plant uptake and which due to removal from the test area due to water flow.

Agrostis stolonifera, the ubiquitous grass species in the region, surviving in all areas except those most affected by smelter plume deposition, shows the lowest ability to concentrate metals of any species tested. This ability to keep metals out of both root and shoot indicates a protective ability. When the metal accumulating data was considered it was suspected that the plant might have a mycorrizal association that inhibited the metal uptake. However, when plants were examined at Okanagan College and University by Dr. D. Durrall, there was no evidence of mychorizae.

4.0 Conclusions

The highest metal uptake for all species across all treatments occurred in controls. However, most were dead or exhibited only poor growth. What could be happening in control plants is a 'soda straw' effect in which plants take up large amounts of metals from their surroundings simply because soluble metals are present in soil water and are therefore available. This severely limits their ability to grow or results in death. In examining the results for control plants it is important to consider Al loading, high levels of which are phytotoxic. Besides diluting metal concentration one effect of the addition of the biosolid residual is to increase the pH of the soil. The reduced levels of Al found in plants grown in treatments other than controls is an indication that low pH could be a contributing factor to plant death in controls.

Harvests were made after one and two months growing time. In the case of controls, due to early death, plants were often harvested sooner. Results for plants in control plots are best used as indicators of a plant=s potential to sequester metals B corroborated by results in treatments.

In general no plants approached the desired level that defines a hyperaccumulator for Zn, Pb or Cd. Levels set for As that would characterize a plant as a hyperaccumulator have not been reported. However, a level of 0.01% has been suggested and this level was used in our research (McIntyre, 1999). No plant investigated in this research achieved this level of accumulation for As. The obtained results do not eliminate the potential shown by some of these plants. Four individual plants several showed marked promise by accumulating better than 0.6% dry weight total for the four metals.

Geranium was not one of the highest accumulators (Table 4) and was surpassed by many other plants. The species exhibited a very large range of metals sequestered. This species included the specimen with the highest level and many of those with very low values (18 of lowest 25) that could be attributed to extreme soil heterogeneity or cultivar differences (two sources of plant used). Due to this wide variability and the concentration of many samples at the lowest end of sequestration levels, the mean value for geranium is substantially lower than the highest potential indicated. But, it produces a large biomass and also has an ability to survive in a low pH environment.

Screening at the University of Guelph had indicated that Mizuna had high phytoremediation potential. This was confirmed in field trials. Shoigun has a large potential root storage organ in its tuber, field results indicated that the plant divides the metals almost equally between root and shoot and that total metal concentrations are high. Cime di rapa broccoletti (*Brassica rapa*) was the best plant in terms of its tissue metal concentrations, however, it produced a lower amount of biomass than many other plants (Table 1) as it was not always harvested when peak biomass was produced. If irrigated, it could easily produce more than one crop in the Trail environment and therefore, it will be included in future investigations. *Eruca sativa* (Arugula) was good at extracting metals and it had shown an ability to survive in high metal concentrations when tested at the University of Guelph. The growing period for this plant is relatively short and it could also produce multiple crops in a single growing season in the Trail region.

The local grass species used in this research, *Agrostis stolonifera* (redtop) grows well throughout the area indicating drought tolerance and ability to withstand an environment in which soil pH is low and metals are present. It had been found in areas where water high in Cd and Zn leaching through a landfill site seeps from a hillside. However, as can be seen in the tables, it is not an accumulator of metals. While metal tolerant it does not sequester large amounts. Of all species tested it demonstrated the least ability to take up metals. For the cumulative total of the four metals the mean value (n= 24) was only 951.08 Fg/g. Its ability to grow well in low pH, high metal environments indicates a potential use for this species in areas where phytostabilization, not metal uptake is the aim of the remediation process.

Field studies showed soil metal concentrations are reduced in all treatments over the 8-week experimental duration. Additionally, several of the plants tested are capable of sequestering relatively large amounts of metals in their tissues. While no plants could be considered as hyperaccumulators several plants were able to accumulate high levels of metals based on plant concentrations. Four plants are considered worthy for further study (*Eruca sativa,* Mizuna, Cime de rapa broccoletti, and geranium). For the fourth plant (geranium) the results obtained in this report suggest that it is perhaps only average in its abilities to concentrate metals. However, due to the extreme variability in size of plants originally transplanted and that some of the plants achieved truly large biomass proportions in comparison to all other plants, they will be used in further testing as well as the other three. The intent of the preliminary work was to establish protocols, assess various plant capabilities and to ensure that subsequent work was based on understanding the particularities of the site=s growth environment. This was done and as a result a second year=s study with an experimental design based on these results was completed during the summer of 1999.

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6.0 References

Baker, A.J.M., Reeves R.D. and S.P. McGrath, In Situ-Decontamination of Heavy Metal Polluted Soils Using Crops of Metal-Accumulating Plants B A Feasibility Study, in *In Situ Bioreclamation* eds. R.E. Hinchee and R.F. Olfenbuttel, Butterworth-Heinemann, Boston MA., pp: 600 B 605, 1991

Campbell C.R., and Plank C.O., Preparation of Plant Tissue for Laboratory Analysis in *Handbook of Reference Methods for Plant Analysis*, eds. Yash. P. Kalra, Soil and Plant Analysis Council, Inc., CRC Press, Boca Raton USA, pp: 37-49, 1996.

Dushenkov, V., Nanda Kumar, Motto H. and I. Raskin, Rhizofiltration: The Use of Plants to Remove Heavy Metals from *Aqueous Streams, Environmental Science and Technology*, Vol. 29, pp: 1239 B 1245, 1995

McIntyre T., Development of a Worldwide Data Base on Metal Tolerant and Metal Accumulating Plants for Contaminated Site Remediation@, in *Proceedings of the Phytoremediation Technical Seminar,* Calgary Alberta, May 31 B June 1 1999, pp: 33-59, 1999

Salt D.E., Blaycock, M. Kumar, N.P. B.A., Dushenkov, V., Ensley, B.D., Chet, I., and I. Raskin, Phytoremediation: A Novel Strategy for the Removal of Toxic Metals for the Environment Using Plants, *Biotechnology* Vol. 13, pp: 468-475,1995,

Saxena, P. K., and Sankaren, Krishnaraj, On the >Scent= of Soil Remediation: Scented Geraniums for Phytoremediation of Metal-Contaminated Soils, *PBI Bulletin, National Research Council of Canada*, 1998.

Soong, Y.K., Determination of Cadmium, Chromium, Cobalt, Lead and Nickel in Plant Tissue, *Handbook of Reference Methods for Plant Analysis*, eds. Yash. P. Kalra, Soil and Plant Analysis Council, Inc., CRC Press, Boca Raton USA, pp: 193 - 198, 1998.