DOE/PC/95701--T3

QUARTERLY TECHNICAL PROGRESS REPORT

DOE-USDA INTERAGENCY AGREEMENT DE-AI22-95PC95701

"Identification and Validation of Heavy Metal and Radionuclide Accumulating Terrestrial Plant Species"

Progress Report for Period of June 21, 1995 - September 20, 1995

PI: Dr. Leon Kochian
U.S. Plant, Soil, and
Nutrition Laboratory, USDA-ARS
Cornell University
Ithaca, NY 14853

Postdoctoral Associates: Dr. Danielle Brady
Dr. Mitch Lasat

Predoctoral Student: Stephen Ebbs

1. Alleviation of heavy-metal induced micronutrient deficiency through foliar fertilization.

As was reported in a previous progress report (for period from 3/20/95 to 3/20/95), we have been attempting to use foliar Fe and Mn sprays to alleviate deficiencies of these micronutrients in plants grown hydroponically in the presence of heavy metals. Two commercial *Brassica* species to be used in the field trial, *B. rapa* and *B. napus*, were grown for seven days in full strength Johnson's solution prior to the addition of 100 μM Zn and 5 μM Cu. Following the appearance of symptoms of chlorosis, various sprays containing either chelated or non-chelated Fe and Mn were applied foliarly twice per week. There were five treatments and two controls (one with no metals in solution and no foliar treatment, the other with metals in solution and no foliar treatment). Plants were harvested after 3 weeks and analyzed for heavy metals. Results for *B. rapa* were submitted in a previous report. Results for *B. napus*. are now available (Table 1).

Table 1. Mean metal accumulation by 4-week old *B. napus* seedlings following foliar fertilization with the treatments below. Values in parentheses represent the range of accumulation for each metal.

Treatment group	mean dry weight, g	[Zn], μg/g	[Cd], μg/g	[Cu],μg/g
no metals, no spray	1.47	170 (83.2-314.2)	0.13 (0.09-0.19)	3.0 (2.2-4.0)
metals, no spray	0.14	1173 (777.6-1467.2)	7.03 (6.07-7.87)	40.2 (32.6-44.0)
$MnSO_4$	0.16	1181 (823.1-1542.0)	7.88 (6.59-11.65)	41.1 (25.7-84.0)
Mn-EDTA	0.13	1185 (746.8-1370.0)	7.17 (6.34-8.29)	30.4 (27.9-32.9)
Fe-EDDHA	0.21	628 (723.5-806.7)	3.86 (3.97-6.19)	18.8 (18.0-32.1)
Fe-EDDHA & $MnSO_4$	0.14	864 (661.0-1117.4)	5.64 (4.91-6.78)	26.4 (19.9-42.1)
Fe-EDDHA & Mn-EDTA	0.21	790 (577.0-955.7)	5.94 (3.25-10.88)	34.1 (19.1-72.2)

The results obtained for *B. napus* are similar to those obtained for *B. rapa*. With only a single exception, plants receiving sprays containing Fe-EDDHA accumulated less Zn, Cd, and Cu than plants receiving sprays with Mn. Chlorosis was alleviated to varying extents by the sprays, with plants receiving both Fe and Mn greening up better than plants receiving either micronutrient alone. The data from *B. napus* also suggest that foliar fertilization with Mn alone appears to more effective at improving metal accumulation than foliar feeding with Fe or Fe in combination with Mn. There was, however, no growth response to the foliar sprays.

The effect of foliar Fe on heavy metal accumulation may be related to the activity of the Fe-deficiency inducible root ferric reductase system. This system is involved in Fe uptake and may also play a role in the uptake of divalent micronutrient cations (like Zn and Cu). Our lab has shown that this reductase is not specific for Fe, in that it is induced by Cu deficiency, and can reduce Cu(II) and Mn(III) ions. When this reductase system was induced by Fe deficiency in peas, there was a dramatic stimulation in the long-term accumulation of several divalent cations, including Zn^{2+}, Cu^{2+}, and Mn^{2+}. Recent studies with radiotracers (^{109}Cd and ^{65}Zn) have shown

that when this reductase is induced, unidirectional Cd^{2+} and Zn^{2+} influx is increased 3-5 fold. The results of the above study likewise suggest that alleviating Fe deficiency with foliar sprays may lead to reduced heavy metal accumulation.

With a preliminary assessment of our micronutrient foliar sprays, we repeated this experiment focusing on fewer foliar treatments but with better replication in the study design. The main goals of this experiment were to confirm the effect of Fe on heavy metal uptake observed in the previous experiment. We had initially planned to spray the field experiment with a combined Fe-EDDHA and Mn-EDTA spray. In light of the previous results with foliar sprays, our recommendation to include Fe in the sprays for the field needed to be reevaluated. Another goal of this experiment was to evaluate the effectiveness of a commercial form of Fe-EDDHA, called Sprint 138. Based on our calculations of the volume of spray required to treat the field site twice per week, the cost of using lab grade Fe-EDDHA was prohibitive. Rather than use a cheaper, less stable chelate such as Fe-EDTA, we chose to use Sprint 138 as a cost efficient alternative. The effectiveness of this commercial formulation in a foliar spray needed to be tested, though.

The protocol was similar to the above experiment with a few modifications. *B. rapa* (cv. Parkland) was the only species tested in this experiment. Only three foliar sprays were tested, a Mn-EDTA spray, a Sprint 138 spray, and a combined spray. Several seeds were germinated in each pot, thinned to two plants per pot after emergence. Plants were grown for 10 days before the metal treatment (100 μM Zn plus 5 μM Cu) was imposed. Plants were sprayed twice per week following the onset of symptoms of chlorosis.

As with the Fe-EDDHA foliar treatment, plants sprayed with Sprint 138 did appear to accumulate less Cd and Cu, but Zn accumulation was essentially the same (Table 2). The highest levels of heavy metal accumulation, though, were observed for plants receiving both Sprint 138 and Mn-EDTA. Unfortunately, due to problems with the cooling system in our greenhouse, the plants most likely experienced heat stress as temperatures in the greenhouse climbed to more than 30 ° C on several occasions. Plants grown in the presence of heavy metals were severely stunted and most began to flower before the end of the three-week growth period. Although the foliar sprays alleviated the chlorosis, they had no effect on shoot growth.

Table 2. Mean heavy metal accumulation by 4-week old *B. rapa* seedlings following foliar fertilization with the treatments below. Values in parentheses represent the range of accumulation for each metal. Sprint 138 is a commercial form of Fe-EDDHA.

Treatment group	mean dry weight, g	[Zn] μg/g	[Cd] μg/g	[Cu] μg/g
no metals, no spray	3.00	50.2 (26.9-83.3)	1.2 (0.9-1.6)	7.0 (5.9-8.7)
metals, no spray	0.08	619.2 (497.8-734.1)	8.5 (6.3-11.4)	46.1 (29.9-76.7)
Mn-EDTA	0.10	659.0 (185.1-1035.8)	8.4 (2.3-13.0)	38.3 (15.2-50.6)
Sprint 138	0.10	700.9 (477.9-1063.8)	6.9 (4.6-10.3)	33.6 (20.9-42.9)
Mn-EDTA + Sprint 138	0.09	692.6 (452.6-927.6)	9.3 (5.1-12.2)	50.6 (28.4-77.5)

2. Second screen for Zn, Cu, and Cd accumulation.

In our previous progress report (for period from 3/20/95 to 3/20/95) we described our the protocol for our second bulk hydroponic screen for heavy metal accumulation. Dicots (mainly selected *Brassica* species) and a number of grasses (including the *Agrostis* and *Festuca* species to be used in the field trials) were grown in 200 L tanks containing full strength Johnson's solution. Plants were grown for 7 days before the heavy metals were added (100 μM Zn, 1 μM Cd, and 5 μM Cu). Following the appearance of symptoms of chlorosis, plants were sprayed twice weekly with a combination of Fe-EDDHA and Mn-EDTA. When our previous report was submitted, results were not yet available. Since that time, analyses have been completed.

The grasses responded well to the foliar sprays, greening up very quickly and maintaining their color throughout the experiment. The impact of the metals on biomass production was less pronounced for some species (*Avena sativa*, *Hordeum vulgare*, *Agrostis tenuis*, *Agropyron elongatum*), more pronounced for others (*Poa compressa*, *Elymus triticoides*, *Elymus cinereus*) (Table 3.). In some cases, the grasses grown in the presence of metals produced more biomass than grasses in the control tank (*Elymus junceus*, *Festuca megalura*, *Eragrostis curvula*, *Puccinela distans*). The growth response displayed by these four species, though, was not necessarily indicative of increased heavy metal accumulation. *Elymus junceus* proved to be one of the better accumulators of Zn and Cd. On the other hand, *Festuca megalura*, *Eragrostis curvula*, and *Puccinela distans* had only marginally greater heavy metal accumulation, more suggestive of the ability to exclude heavy metals, rather than accumulate them. The best heavy metal-accumulating grasses from this screen are shown in Table 4. Heavy metal accumulation for these species is presented as both concentration in the plant tissue (μg heavy metal per gram tissue) and as total uptake (total μg heavy metal). Since the biomass of individual plants of some grass species is extremely small, the latter value incorporates plant biomass in order to more accurately reflect the removal of heavy metals from the nutrient solution.

Table 3. Mean per plant dry weights for various grass species grown in the presence of 100 μM Zn, 1 μM Cd, and 5 μM Cu as compared to controls.

species	dry weight of control plants, g/plant	dry weight of heavy metal-grown plants, g/plant
Avena sativa	2.120	2.103
Hordeum vulgare	3.498	1.023
Agrostis tenuis	0.031	0.026
Agropyron elongatum	0.344	0.183
Poa compressa	0.046	0.004
Elymus triticoides	0.068	0.017
Elymus cinereus	0.109	0.046
Elymus junceus	0.145	0.190
Festuca megalura	0.012	0.044
Eragrostis curvula	0.022	0.178
Puccinela distans	0.026	0.070

Table 4. Mean heavy metal concentration (μg/g) and total metal uptake (μg) by selected grass species. Total uptake refers to the total amount of heavy metals removed by the specified number of plants during the 4-week growth period.

			Zinc		Cadmium		Copper	
Grass Species	mean dry weight, g	total # plants	tissue conc.	total uptake	tissue conc.	total uptake	tissue conc.	total uptake
Avena sativa	2.103	1	957.0	2012.5	18.2	38.2	22.9	48.2
Hordeum vulgare	1.023	10	1478.9	23563.7	17.6	253.4	39.4	78.0
Agropyron elongatum	0.183	17	1240.5	3789.3	61.7	176.7	32.2	96.0
Elymus junceus	0.190	10	1311.2	2198.7	52.7	88.7	23.4	40.0
Festuca rubra	0.256	27	309.9	180.3	6.8	4.0	32.0	18.6
Agrostis capillaris	0.120	10	579.9	69.6	17.4	2.1	38.2	4.6

Surprisingly, oats (*Avena sativa*) and barley (*Hordeum vulgare*) performed well, producing good biomass when treated with heavy metals. Barley removed nearly 10 times more Zn than the next best species for Zn uptake, *Agropyron elongatum*, because it produced 10 times greater biomass. *A. elongatum* showed the greatest total Cu uptake, though, with barley taking up somewhat less. Although the data for *A. sativa* is quite impressive, it is based up the growth and metal uptake by a single plant. The performance of *A. sativa* thus needs to be verified in additional experiments. Despite evidence of heavy metal accumulation from field survey data, the two grass species that were to be tested in the field trial (*Festuca rubra* and *Agrostis capillaris)* proved to be among the poorest heavy metal accumulators in this screening experiment.

In terms of leaf chlorosis, the dicots also responded well to the foliar treatments, with much of the chlorosis being alleviated by the foliar sprays. All the dicots, though, displayed a significant reduction in growth when exposed to the combination of heavy metals, ranging from a 13% reduction in growth for *B. oleracea* to a 76% reduction for *B. nigra*. All other Brassicas showed a growth reduction of 40 to 65%. The growth reduction for *Ipomea* was only 19%. As in our first heavy metal screen, all the *Brassica* species tested accumulated comparable levels of heavy metals (Table 5). There was, however, considerable variability in the data, as indicated by the large standard deviations associated with the means. Since the means for each Brassica species include data from several different accessions of that species, the variability may in part reflect the genotypic variation in heavy metal accumulation within the species. *B. rapa* and *B. napus* consistently showed the greatest metal uptake among the Brassicas, due in part to the slightly greater biomass produced by these species as compared to the other *Brassica* species. The two species of *Ipomea*, also tested in the first screening experiment, showed comparable Zn and Cd uptake. For Cu, the *Ipomea* species took up nearly twice as much Cu as *B. rapa*.

To verify the performance of the species discussed above, an upcoming experiment will compare three grass species (*A. sativa*., *H. vulgare*, and *A. elongatum*) to two accesions of *Brassica juncea* and one accession of *B. rapa*.. Plants will be grown for 10-14 days before one of two heavy metal treatments (no metals or 100 μM Zn plus 1 μM Cd) is imposed. As symptoms of chlorosis appear, a subset of plants from each heavy metal treatment will be foliarly treated with a spray containing Fe-EDDHA and Mn-EDTA. Fe-EDDHA will be included in the foliar spray as Sprint 138 (see above), allowing an opportunity to test the effectiveness of this form of Fe formulation

for foliar feeding. In addition, spraying plants grown in the absence of heavy metals will allow us to determine if there is any detrimental effect of the foliar spray itself. Plants will be allowed to grow for 4+ weeks before they are harvested for analysis. From this experiment we should be able to directly compare the effectiveness of these two groups of plants for phytoremediation and demonstrate whether one or more grass species are likely to be good candidates for field trials next year. By comparing sprayed grasses to unsprayed grasses, we will also be able to determine if foliar Fe has an effect on heavy metal uptake by grasses.

Table 5. Mean heavy metal concentration (μg/g) and metal uptake (μg) by *Brassica* and *Ipomea* species. Data for the Brassica species represent the mean values for all accessions of that species. Data for *Ipomea* represent the mean values for *Ipomea purpea* and *Ipomea hederacea*. Values in parentheses indicate standard deviations from the mean.

		Zinc		Cadmium		Copper	
Species	mean d.w., g	tissue conc.	total uptake	tissue conc.	total uptake	tissue conc.	total uptake
B. juncea	0.51	1355.0 (409.9)	570.4 (398.3)	29.5 (8.4)	12.9 (9.5)	18.7 (5.4)	9.0 (8.7)
B. napus	0.72	1270.1 (279.1)	875.3 (547.8)	29.2 (7.8)	21.3 (16.7)	18.8 (3.8)	13.6 (10.4)
B. nigra	0.41	1244.8 (262.6)	479.6 (242.8)	26.6 (5.9)	10.2 (5.0)	19.9 (7.5)	7.2 (3.7)
B. oleracea	0.61	870.0 (97.2)	551.9 (476.4)	21.0 (1.7)	12.4 (9.1)	19.3 (2.1)	11.1 (7.3)
B. rapa	0.86	1244.7 (248.6)	1002.0 (620.2)	28.2 (4.9)	23.7 (15.9)	21.9 (10.5)	17.5 (12.0)
Ipomea spp.	1.62	426.6 (70.1)	692.8 (134.7)	6.7 (0.4)	10.8 (0.3)	19.0 (5.7)	30.6 (8.2)

3. Characterization of the root Zn hyperaccumulation by *Thlaspi caerulescens*

Parallel with the current field work at the sites in Montana and Idaho, our group began more basic research. The purpose of this research is to identify the mechanism(s) responsible for Zn hyperaccumulation in *T. caerulescens*.

Although *T. caerulescens* has been shown to accumulate up to 3% Zn dry weight in the shoots, this species produces only a small biomass, making it unsuitable for practical use in phytoremediation. However, if the mechanism responsible for Zn hyperaccumulation could be transferred to a higher biomass plant species, then an ideal candidate would be obtained. Previous work suggested that the heritability of hyperaccumulation maybe simple, involving only one or a few genes. If this is the case, it is likely that existing molecular biology techniques could be used to transfer this trait to a different species. Before this can be accomplished, the mechanism of hyperaccumulation must be identified..

Two possible mechanisms may be involved in the ability of *T. caerulescens* to accumulate high levels of Zn : 1) greater absorption of Zn by roots; 2) enhanced translocation from roots to shoots. Currently, we have been comparing these putative mechanisms in *T. caerulescens* to a nonaccumulator species, *Thlaspi arvense*. Once we determine the mechanism(s) involved, molecular biology techniques will be employed to identify the genes associated with these mechanisms.

4. Comparison of commercial *Brassica* accessions to *Brassica* accessions obtained from the Iowa State seed bank

Although we have obtained *Brassica* seed from the collections held at Iowa State, we do not have enough seed remaining to begin a comprehensive series of experiments. Likewise, for *B. juncea*, we have only a small amount left of the seed provided by Dr. I. Raskin (Rutgers University). Since we would prefer not to delay our work until a seed increase can be accomplished, the alternative was to test commercial varieties of Brassicas in hopes of finding ones with the same ability to accumulate and tolerate heavy metals. This experiment compared the *B. juncea* obtained from Dr. I Raskin (accession number 426308), a *B. rapa*, and a *B. napus* to commercial varieties of the same species obtained from a supplier in Canada.

This experiment was useful in diagnosing which metals were responsible for the micronutrient deficiency and inhibition of root growth that we have observed in past experiments. There were three heavy metal treatment groups in this study - Zn only (100 μM), Cu only (5 μM), and Zn+Cu (100 μM+5 μM) and a control group (no metals). From the single heavy metal groups, we were able to observe differing phytotoxic responses, which were then be compared to the corresponding control and the Zn+Cu treatment. Cu was expected to be the element primarily responsible for the inhibition of root growth while both Zn and Cu were thought be responsible for the chlorosis and the micronutrient deficiency. Quantifying the effects of the heavy metals on several growth parameters was of paramount importance in this study. The following parameters were measured in this study: shoot and root dry weight, length of main root, density of primary lateral roots (# primary lateral roots/cm tap root), and chlorophyll content of both old and young leaves. Chlorophyll measurements were made using a Minolta Spad-502 chlorophyll meter. In addition to diagnosing the deficiency, this experiment should provide valuable background information on which to base further foliar feeding experiments.

Seeds of the eight *Brassica* accessions were germinated on filter paper and transferred to 5 L pots after 24-36 hours. Each pot, which contained a full-strength Johnson's solution,. had one plant of each species and accession, for a total of 8 plants per pot (i.e.- four *B. junceas*, two *B. rapas*, etc.). The four treatments groups in this study were a control group (no metals), a Zn only group (100 μM), a Cu only group (5 μM), and a Zn+Cu group (100 μM+5 μM). Each treatment was replicated 6 times. Plants were grown in the pots for 7-10 days before the metal treatments are imposed. Plants were grown for 2 weeks after the metal treatments were imposed with chlorophyll content of the first fully-expanded leaf and the youngest leaf measured at 2 day intervals. Heavy metal treatment with Cu and, to a lesser extent, Zn, induced flowering in most plants, especially in *B. rapa*. At the termination of the experiment, shoots were harvested, separated into old and young portions, dried, and digested for ICP analysis. Roots were also harvested, with measurements of primary lateral root density made for all species before ICP analysis. Heavy metal analyses for the commercial *B. juncea* and the *B. juncea* provided by Dr. I. Raskin have been completed and analyses of the remaining species are currently underway.

As was expected, Cu showed the most dramatic effect on the development of lateral roots (Table 6). For all accessions except the commercial *B. juncea* and *B. juncea* 184290, 5 μM Cu

significantly decreased the density of primary lateral roots. The same generally held true for Cu in conjunction with 100 μM Zn. The two accessions of *B. napus* and only one accessions of *B. juncea* (459007) appeared to be the only ones adversely affected by 100 μM Zn. The commercial *B. juncea* and *B. juncea* 184290 appear to be less sensitive to these metals, either alone or in conjunction, than the other accession or species tested. These species actually had slight greater primary lateral root densities when grown in the presence of 100 μM Zn than control plants. The same held true for *B. rapa* 163496 in the presence of Zn.

Table 6. Mean primary lateral root density (# primary laterals/cm tap root) for the eight *Brassica* accessions grown for 10 days in the presence of 100 μM Zn, 5 μM Cu, or 100 μM Zn+5 μM Cu. Numbers following species names refer to the accession number of that species. The abbreviation 'comm.' indicates a commercial variety of the given species.

Metal treatment	*B. juncea* (comm.)	*B. juncea* 426308	*B. juncea* 459007	*B. juncea* 184290	*B. rapa* (comm.)	*B. rapa* 163496	*B. napus* (comm.)	*B. napus* 535855
control	4.41	5.41	5.64	3.56	9.34	6.01	6.76	8.30
Zn	4.85	3.51	3.67[a]	4.14	7.22	6.54	3.88[b]	5.28[a]
Cu	2.63	2.78[a]	2.21[a]	2.59	5.11[b]	3.43[a]	2.32[b]	3.19[b]
Zn+Cu	2.99	2.55	2.63[a]	2.19	3.78[b]	3.03[a]	2.00[b]	3.27[b]

[a] = significantly less than control at $\alpha = 0.05$
[b] = significantly less than control at $\alpha = 0.01$

Symptoms of chlorosis appeared within all species 2-3 days following the imposition of the metal treatment. Chlorophyll measurements were initiated at that time. Some of the accessions tested, particularly the accessions of *B. juncea*, appeared to tolerate the metals, especially Cu, quite well, with some leaves showing no evidence of chlorosis. In fact, some leaves had more chlorophyll than the corresponding control leaves (Table 7). As the treatment period progressed, differing patterns of chlorosis appeared in plants receiving the different heavy metal treatments. Plants grown in the presence of 100 μM Zn showed chlorosis exclusively in the younger leaves, with chlorosis and necrosis appearing in the older leaves only near the termination of the experiment. Chlorosis in plants grown in the presence of Cu started in the older leaves and move progressively up the plant until all leaves displayed some degree chlorosis. For the Zn+Cu treatment, only *B. rapa* and *B. napus* exhibited leaf chlorosis. Chlorophyll levels in the oldest leaves decreased the most, with the younger leaves tending to have slightly more chlorophyll than the corresponding control plants. *B. juncea* plants generally showed no reduction in chlorophyll content when grown in this treatment, tending again to have a slightly greater chlorophyll content than control plants.

Heavy metal uptake and accumulation by roots and shoots was comparable for the commercial versus the Raskin *B. juncea* (Tables 8-9). Whereas the roots of *B. juncea* 426308 tended to take up slightly more Zn that the roots of the commercial variety, the reverse was true for Cu. Likewise, *B. juncea* 426308 produced a slightly greater shoot biomass for all treatments, while the commercial variety had slightly greater root biomass. Analyses of the shoots from both species revealed that *B. juncea* 426308 took up approximately 2.5 times more Zn than the

commercial variety but about the same amount of Cu. Micronutrient (Mn and Fe) levels in the shoots of both are species are lower than the corresponding control plants, consistent with the heavy metal-induced deficiency observed in previous experiments.

Table 7. Relative chlorophyll content, expressed as percent of control, of old and young leaves from different accessions of *Brassica* plants grown in the presence of 100 μM Zn, 5 μM Cu, or 100 μM Zn+5 μM Cu.

	100 μM Zn		5 μM Cu		100 μM Zn+5 μM Cu	
species & accession	leaf #1	leaf #3	leaf #1	leaf #3	leaf #1	leaf #3
B. juncea, commercial	89.0	46.2	130.9	119.7	138.5	131.3
B. juncea, 426308	81.0	59.5	78.6	107.4	80.8	135.3
B. juncea, 459007	69.7	82.3	123.7	121.1	110.4	139.8
B. juncea, 184290	111.5	89.0	110.3	88.4	118.2	133.3
B. rapa, commercial	96.1	74.7	4.7	118.6	21.8	122.2
B. rapa, 163496	108.6	70.7	25.5	124.2	34.2	124.0
B. napus, commercial	54.6	39.8	74.7	87.3	84.3	88.9
B. napus, 535855	64.5	31.6	89.2	64.1	77.6	91.0

Table 8. Micronutrient and heavy metal uptake and concentration in the roots of a commercial (comm.) *B. juncea* and *B. juncea* 426308 grown in the presence of 100 μM Zn, 5 μM Cu, or 100 μM Zn+5 μM Cu.

			uptake, μg				concentration, μg/g			
accession	treatment	dry wt.,g	Mn	Fe	Zn	Cu	Mn	Fe	Zn	Cu
commercial	control	0.077	0.55	5.8	5.0	1.3	7.1	71.1	66.6	17.5
	Zn	0.021	0.28	14.0	124.7	2.1	13.8	675.4	6070.2	105.2
	Cu	0.059	0.86	41.2	6.9	52.5	14.8	746.9	109.4	850.3
	Zn+Cu	0.047	0.60	25.9	114.6	45.1	13.1	543.0	2424.9	921.0
426308	control	0.222	1.86	15.1	16.2	3.5	8.5	68.4	75.6	16.4
	Zn	0.039	0.76	19.6	275.4	4.5	18.4	562.3	6535.6	107.6
	Cu	0.037	0.46	12.7	4.1	30.6	14.4	394.1	107.5	800.7
	Zn+Cu	0.032	0.36	19.9	72.3	29.1	11.4	610.5	2307.6	903.0

Table 9. Micronutrient and heavy metal uptake and concentration in the shoots of a commercial (comm.) *B. juncea* and *B. juncea* 426308 grown in the presence of 100 μM Zn, 5 μM Cu, or 100 μM Zn+5 μM Cu.

			uptake, μg				concentration, μg/g			
accession	treatment	dry wt.,g	Mn	Fe	Zn	Cu	Mn	Fe	Zn	Cu
commercial	control	1.057	32.6	54.7	34.7	6.1	30.2	50.9	32.8	5.9
	Zn	0.470	3.9	8.9	398.0	2.6	9.0	18.3	850.6	6.5
	Cu	0.263	4.9	7.0	8.1	8.0	19.5	27.6	31.4	30.8
	Zn+Cu	0.181	2.7	7.1	57.3	5.1	14.5	40.7	325.5	28.6
426308	control	1.880	14.3	87.0	64.0	14.3	8.0	41.2	31.8	8.0
	Zn	0.537	10.1	17.2	914.1	12.9	15.2	26.3	1344.5	18.3
	Cu	0.306	4.1	7.1	8.1	8.6	14.3	26.0	27.3	31.2
	Zn+Cu	0.227	3.7	8.7	73.5	6.2	16.8	40.2	363.2	28.9

5. Second screening experiment for the accumulation of Cs and Sr by plants.

The most promising species of Brassica and legumes from the first Cs/Sr screening experiment were tested again to confirm their ability to accumulate Cs and Sr. The Brassicas tested included two varieties of *B. juncea*, and one variety of *B. napus*, *B. rapa*, and *B. carinata*. A commercial broccoli ("Saga") and cabbage ("Storage #4") were also tested. In the first screening experiment, this variety of cabbage was the best accumulator of Cs and Sr among the Brassicas tested. Legumes, however, performed even better in that experiment than the Brassicas. Thus, a variety of *Vicia sativa*, *Vicia villosa*, and three varieties of *Phaseolus accutifolius* were retested. Four grass species retested were *Poa sandbergii*, *Agrostis alba*, *Agrostis capillaris*, and *Festuca rubra*. Field survey data for the later two species initially suggested that these species were heavy metal accumulators and might be considered candidates for Cs and Sr accumulation. However, data from the second heavy metal screening experiment (discussed above) showed that these species were, in fact, poor metal accumulators. These two species showed moderate Cs and Sr accumulation in our first experiment, though, but with a fairly low biomass. They were included in this second Cs/Sr screen in hopes of improving both growth and radionuclide accumulation. Additional species included in the rescreening experiment at the request of MSE, Inc., were *Vicia faba*, *Medicago sativa*, and *Helianthus annus*.

Depending on the species, seeds were germinated for 1-3 days before being transferred to pots containing a modified full strength Johnson's solution with 20 μM Sr and 1 μM Cs. Due to some germination and early growth problems, seedlings from some species (*H. annus*, *P. sandbergii*, *B. carinata*, *B. nigra*, and *B. rapa*, either died or grew so poorly that they were replaced with seedlings of other species in this experiment. Plants were harvested after 3-4 weeks and analyzed for Sr. Analyses for Cs have yet to be completed.

All the species screened, with the exception of *Medicago sativa* and *Festuca rubra*, accumulated Sr fairly well (Table 10). *P. accutifolius*, *V. faba*, *V. villosa*, *B. juncea*, and cabbage, storage #4 were by far the best accumulators, taking up nearly 100 μg of Sr during the growth period. Although Sr accumulation by *V. sativa* was considerably less than what was observed in the first screening experiment (144 μg vs. 49.7 μg in this experiment), the other two results from *V. villosa* and *V. faba* suggest that these vetch species may be fairly effective at removing Sr from soils. The results from the Brassica species and from *P. accutifolius* likewise confirm that these species are capable of accumulating Sr from solution and, thus, may be suitable for use in the field. The results for *A. capillaris* may also appear encouraging but most likely do not represent the true ability of that species to accumulate Sr. Data for *A. capillaris* was extremely variable, with values for Sr uptake ranging from 15.0 to 131.8 μg and tissue Sr concentrations ranging from 65.1 to nearly 3000 μg/g. The highest biomass *A. capillaris* plant (0.231 g) had the lowest Sr uptake and accumulation while the lowest biomass plants (0.044 and 0.064) had the greatest. This suggests that the results obtained may reflect a concentration effect rather than the ability of this species to accumulate Sr. Based on the results from these two screening experiments, dicots appear to be the most promising species for removal of Sr from contaminated soil. Once the results for Cs accumulation are available, it will be possible to select species capable of accumulating both elements for use in field trials.

Table 10. Mean uptake and concentration of Sr in plants grown in the presence of 20 μM Sr and 1 μM Cs. Numbers following the names of some species refer to the accession of that species used in the experiment.

species	# plants	mean dry weight, g	Sr uptake, μg	[Sr], μg/g
P. accutifolius L177	1	0.181	160.4	886.3
V. faba	6	0.310	106.8	348.0
M. sativa	7	0.046	19.5	411.7
V. villosa	7	0.196	89.4	462.9
V. sativa	8	0.124	49.7	390.3
cabbage, storage #4	9	0.112	89.3	791.0
broccoli, Saga	5	0.050	38.5	774.5
B. carinata 360881	1	0.163	67.1	412.0
B. nigra 367904	1	0.153	24.0	156.5
B. rapa 164468	1	0.105	52.7	502.1
B. juncea 459007	6	0.186	93.6	552.3
B. napus 535873	10	0.105	54.3	522.5
A. alba	4	0.126	60.4	481.1
A. capillaris	5	0.135	68.3	1014.1
F. rubra	5	0.036	11.1	292.6

6. Effect of Ca on Cs and Sr accumulation by selected dicot species.

Since Ca^{2+} and Sr^{2+} are analogs and most likely enter plant roots via the same mechanisms, it is likely that the Ca level in the soil or nutrient solution will influence the extent of Sr accumulation by plants. In the first Cs/Sr screening experiment, the species that showed the greatest Cs and Sr accumulation among the Brassicas (cabbage, Storage #4) had the lowest Ca/Sr ratio among the tested Brassicas. The legumes that accumulated Cs and Sr well (*P. accutifolius*, *V. villosa*, and *V. sativa*) also had among the lowest Ca/Sr ratios. Increasing solution Ca levels could, therefore, compete with Sr for uptake and decrease the removal of Sr. There may also be an effect of Ca on Cs uptake.

To test whether Ca has an effect on Cs and Sr accumulation, four dicot species (*B. juncea*, *B. napus*, *V. villosa*, and cabbage, Storage #4) were germinated for 1-2 days before being transferred to pots containing full strength Johnson's solution. The Ca concentration in the pots was adjusted to give one of two Ca/Sr ratios: 200:1 and 400:1. The former reflects the ratio of Ca in full strength Johnson's solution to 20 μM Sr. Plants were grown for 3-4 weeks before harvest and analyzed for Sr. Cs analyses have yet to be completed.

Surprisingly, the results showed that with the exception of cabbage, plants grown at a Ca/Sr ratio of 400:1 accumulated more Sr than plants grown at a Ca/Sr ratio of 200:1 (Table 11). The same generally held true for Sr tissue concentration. However, again with the exception of cabbage, plants grown with a Ca/Sr ratio of 400:1 were larger than plants grown at the lower Ca/Sr ratio. For *B. napus* and *V. villosa*, the difference in biomass was more than 50%. The reason for this

difference is not known. The biomass values obtained for plants grown at a Ca/Sr ratio of 200:1 are similar to those obtained for these species in our earlier screening experiments. This is not surprising because this ratio reflects the ratio of Ca in a full strength Johnson's solution (4 mM Ca) to the level of Sr in the nutrient solution (20 μM). At a Ca concentration of 8 mM (400:1 Ca/Sr ratio), biomass of *B. juncea*, *B. napus*, and *V. villosa* increased significantly, as did Sr uptake. Thus, it appears that the increased biomass at the higher Ca level resulted in greater transpiration, which, in turn, resulted in greated Sr translocation to the shoot.

Table 11. Effect of Ca/Sr ratio on Sr uptake and accumulation by selected dicot species.

	200:1			400:1		
species	mean dry weight, g	Sr uptake, μg	tissue [Sr], μg/g	mean dry weight, g	Sr uptake, μg	tissue [Sr], μg/g
cabbage, storage #4	0.110	59.0	521.9	0.123	56.4	469.2
B. napus	0.090	34.4	362.8	0.199	55.7	274.7
B. juncea	0.219	71.4	330.2	0.332	123.9	351.1
V. villosa	0.120	25.9	215.8	0.251	81.6	384.7

7. Preliminary investigations into the forms of uranium taken up by plants.

An experiment designed to investigate which forms of U might be taken up by plants from nutrient solution was repeated with greater control of solution chemistry. A solution of depleted $^{235}UO_2(NO_3)_2$ was supplied to a half strength modified Johnson's solution at 5 uM total concentration. Pea (cv Sparkle) plants were precultured in high P solution (100 μM) for one week and then transferred to solutions containing U, but no P, for one week. After modeling of nutrient solutions with GEOCHEM-PC; three treatments were chosen which gave a good separation of U complexes. At pH 5 a significant amount of U was present as a free ion, at pH 6.5 most U was present as hydroxide complexes and at pH 8 nearly all U was present at carbonate complexes. Plants were grown in the absence of U at the test pHs to provide a toxicity control. Observations of roots at harvest confirmed that U is quite toxic to roots with growth being arrested immediately after transfer to U containing solutions.

Uptake of U into the shoots of Sparkle peas was influenced by pH and hence by form of U in solution (Table 12). The greatest uptake occurred at pH 5 when U was present predominantly as a free ion of positive charge (UO_2^{2+}). Uptake at pH 6 was less than 20 % of that at pH 5. At this pH U was present predominantly as negatively charged hydroxide species. We suggest that the small amount of uptake occurred as due to the presence of some free UO_2^{2+} at pH 6. Uptake at pH 8, when 95%+ of available U was present as carbonate species, was very low.

Roots were removed from solution and rinsed in four changes of water before blotting and drying. A desorption procedure was not carried out. Therefore the U concentrations presented for roots are due to absorption and/or adsorption to the roots. Root growth was greatly inhibited by U. Uranium in the roots was greatest at pH 6 and 8 with much lower concentration at pH 5.

The reason for these differences are not known yet, but are probably due to surface exchange properties of the roots, which are influenced by pH.

Table 12. The effect of uranium in solutions of different pH on pea (cv. Sparkle) growth, concentration of U, and total uptake of U. Mean dry weight is reported for plants grown in both the presence (+U) and absence (-U) of uranium. Numbers in parentheses represent standard deviations from the mean.

	roots				shoot			
pH	-U dry wt, g	+U dry wt, g	tissue U, µg/g	U uptake, µg	-U dry wt, g	+U dry wt, g	tissue U, µg/g	U uptake, µg
5	0.15	0.09	552 (123)	48 (8)	0.31	0.34	1.28 (0.41)	0.44 (0.10)
6	0.19	0.05	10351 (856)	551 (80)	0.34	0.39	0.22 (0.07)	0.08 (0.02)
8	0.20	0.06	10051 (1000)	545 (99)	0.36	0.48	0.08 (0.04)	0.04 (0.02)

Although the total uptake rates in this experiment were low (< 1 µg/g U), the plants were only a week old. The differing uptake results for the three pH treatments were highly significant and when confirmed have serious implications for practical phytoremediation. If significant uptake of U only occurs at pHs were it is present as a free ion, many of the U contaminated sites which have been amended with alkaline, carbonaceous materials will not be conducive to phytoremediation. It is likely that soil pH will need to be reduced for significant uptake to the shoots to occur. The results for root adsorption/uptake have important implications for rhizofiltration. Greatest uptake of U from contaminated water will occur when the pH is raised to > 6. The difference in uptake between pH 5 and 6 is over 20 fold. While uncontaminated water may be expected to be around neutral, water contaminated with heavy metals may be lower as hydrolysis of metals such as Al and U leads to acidification of the solution.

In an additional experiment, Sparkle was again used as a test plant in an experiment to determine if U uptake can occur in the presence of phosphate. Preliminary observations suggest that U is much less toxic in the presence of P, but its effects are not completely mitigated. A small screening experiment has also been conducted in minus P solution, using plants precultured at high P. Two species of *B. juncea*, one species of *B. rapa*, beet, hairy vetch, common vetch, alfalfa, Sparkle and the bronze mutant E107 will be compared for U uptake. These two experiments are currently being analyzed.