DOE/PC/95701--T1

QUARTERLY TECHNICAL PROGRESS REPORT

DOE-USDA INTERAGENCY AGREEMENT DE-AI22-95PC95701

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

Progress Report for Period of March 20, 1995 - June 20, 1995

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

In the first screen 285 test species were grown in 200 L tanks of full strength Johnson's solution with 100 μM Zn, 5 μM Cu and 1μM Cd. Dry seed was planted directly onto mesh in wells of a polystyrene float in direct contact with the nutrient solution which contained the heavy metals. In the second screen the same *Brassica* species were pre-cultured for 7 days in full strength Johnson's solution in the absence of heavy metals. A range of grass species were also tested in this experiment, including the *Agrostis* and *Festuca* species to be used in the field trials. Symptoms of chlorosis became apparent on all plants several days after addition of the heavy metals to solution. Analyses of plants from the first screen had shown that the heavy metal treatment induced deficiencies of Fe and Mn. From the first appearance of symptoms, the plants in the second screen were sprayed with a solution of Fe-EDDHA, Mn-EDTA and L-77 as a surfactant in an attempt to alleviate the deficiency. In contrast to the first screen, there was a response to the foliar spraying with plants greening significantly. Plants were grown for 4 weeks and then whole shoots and roots were harvested. Plant analysis is currently being undertaken.

2. Foliar fertilization with Fe and Mn to overcome heavy metal-induced micronutrient deficiency.

The effectiveness of a series of Fe and Mn foliar sprays in alleviating an Fe and Mn deficiency was assessed in a separate controlled experiment. The two cultivars of *B. napus* and *B. rapa* to be used in the field trial were grown in the same culture system as the screening experiments (pre-culture for 7 days in full strength Johnson's solution prior to 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 a week. There were five treatments, shown below, and two controls (one with no metals in solution and foliar treatment, the other control with metals in solution but no foliar spray). Plants were harvested after 3 weeks and analyzed for the heavy metals. The analyses for *B. rapa* have been completed.

The data show that both species showed some metal hyperaccumulation. Based on the results from *B. rapa*, plants receiving sprays containing Fe-EDDHA accumulated less Zn and Cd (Table 1). With only a single exception, the same trend held true for Cu accumulation. The only growth response evident was for plants receiving Fe-EDDHA and $MnSO_4$ (Table 2), but at the expense of metal accumulation. Plants receiving foliar Fe and Mn greened up considerably more than plants receiving either micronutrient alone, and tissue analysis revealed no cation micronutrient deficiencies. From this preliminary data, it appears that foliar fertilization with Mn alone appears to be more effective at improving metal accumulation that foliar feeding Fe or Fe in combination with Mn.

The extent of metal accumulation was much less than what was observed in a previous experiment with these species (Cu/Zn 'tolerance' experiment). One possibility is that by alleviating the Mn and Fe deficiencies, the ability to hyperaccumulate heavy metals has somehow been altered. Perhaps accumulation is related in some way to Fe and/or Mn transport or metabolism. By increasing both solution Mn and Fe while simultaneously applying the elements foliarly, accumulation may have somehow been affected. While discouraging in terms of total metal accumulation, this experiment may shed some light on both the hyperaccumulating ability of this species and the role(s) that Fe and Mn may play in hyperaccumulation.

Assuming the data from *B. napus* follows a similar pattern, the next step would be to repeat this experiment focusing on only a few foliar treatments with better replication. Examining the effects of single metals in solution (Zn only, for instance), may also provide information regarding the nature of the micronutrient deficiency and whether foliar sprays can be used to both alleviate the deficiency while also improving growth and metal accumulation.

Table 1. Mean metal accumulation by *B. rapa* following foliar fertilization with the treatments below. Values in parenthesis represent the range of accumulation for each metal.

foliar treatment	[Zn], µg/g	[Cd], µg/g	[Cu], µg/g
no metals, no spray	308.8 (76.3-587.6)	0.69 (0.04-1.59)	6.6 (0.84-13.8)
metals, no spray	1165.0 (729.8-1519.1)	7.1 (5.5-9.4)	39.0 (19.9-54.0)
$MnSO_4$	1261.6 (843.3-1536.3)	7.4 (4.3-9.0)	40.3 (26.8-51.9)
Mn-EDTA	1390.8 (612.5-1808.7)	8.3 (6.8-10.7)	43.0 (34.4-50.6)
Fe-EDDHA	859.1 (331.2-1206.3)	5.3 (4.4-6.6)	32.7 (30.5-36.5)
Fe-EDDHA & $MnSO_4$	837.5 (704.8-1.95.6)	4.5 (3.9-5.7)	26.1 (16.6-42.9)
Fe-EDDHA & Mn-EDTA	970.5 (690.0-1077.2)	5.7 (3.0-8.3)	63.9 (23.6-160.4)

Table 2. Dry weight of *B. rapa* plants receiving various foliar sprays.

foliar treatment	mean dry weight, g
no metals, no spray	1.18
metals, no spray	0.14
$MnSO_4$	0.18
Mn-EDTA	0.15
Fe-EDDHA	0.14
Fe-EDDHA & $MnSO_4$	0.27
Fe-EDDHA & Mn-EDTA	0.08

3. Screening for Cs/Sr accumulation.

A range of plant species were tested for their ability to accumulate Cs and Sr in shoots, including a number of species from the family, *Brassicaceas* previously identified as potential candidates for the phytoremediation of heavy metal (Zn, Cd, Cu)-polluted soils. In addition, since Cs is an analogue of Na, a variety of halophytes were also tested. Finally, since some legumes are known to accumulate Ca to high concentrations and because Sr is an analogue of Ca, several legume species were also included in the screening.

Filter paper-germinated 3 day-old seedlings were grown hydroponically in 6 L black plastic pots filled with modified full strength Johnson solution containing 20 µM $SrCl_2$ and 1 µM CsCl. The nutrient solution was replaced weekly. Most of the plants including all *Brassica's* were harvested 15 days after being transferred to the hydroponic solutions. However, some of the slower growing grasses and dicots were harvested as late as 45 days after being transferred to the hydroponic. Shoots were analyzed for Ca, Sr, and Cs content.

Dicots appear to have a greater ability to accumulate Cs and Sr in shoots compared with grasses. Among *Brassicaceaes,* a commercial variety of cabbage (Storage N° 4) had the highest rate of accumulation for both metals (Table 3). Interestingly, this variety had the lowest Ca/Sr ratio among the tested *Brassicaceaes.* In terms of total Sr accumulation per plant, for the *Brassica's,* the greatest levels of Sr were accumulated by high biomass producing varieties of *B.napus* (458935) and *B. rapa* (164468). Overall, however, the best performers were legume species. Thus, a variety of *Phaseolus accutifolius* (L166) accumulated as much as 29 and 1820 (µg g dry wt^{-1}) of Cs and Sr, respectively. High rates of accumulation appear to correlate well with low Ca/Sr ratios. Compared with dicot species, grasses accumulated relatively low levels of both Cs and Sr. For Sr, the best accumulation rates were obtained with *Agrostis alba* and *Poa sandbergii.* These rates, however, were only 50% of those observed for *Brassicaceaes.* Interestingly, low Ca/Sr ratios were observed in both species. Finally, the Zn hyperaccumulator, *Thlaspi caerulescens*, does not appear to be a strong candidate for the accumulation of Cs and Sr.

Table 3: Concentration and accumulation of Sr And Cs by selected species.

Species	No of accessions	Cs concentration (µg/g dry wt)	Cs accumulation per plant (µg)	Sr concentration (µg/g dry wt)	Sr accumulation per plant (µg)
Brassica juncea	8	15±2.3	1.4±0.5	365±18	36±11
Brassica napus	6	14±1.4	1.8±0.4	384±12	53±12
Brassica nigra	2	11	1	382	33
Brassica rapa	2	9	1.1	358	47
Cabbage	1	21	2.1	508	50
Phaseolus accutifolius	1	16	5.8	709	272
Vicia sativa	1	15	2.1	1050	144
Thlaspi caerulescens	1	10	0.3	109	4
Agrostis capillaris	1	6	0.4	169	11
Agrostis alba	1	7	0.7	206	21

A future experiment is proposed with the following objectives:

-A careful determination of the Sr and Cs accumulation rates for legume species and few selected grasses and *Brassicaceaes.*
-Test the accumulation rates in *Ph accutifolius* L166 and L177 vs. P1319443 (We found in results not reported that great differences exist between L166 or L177 and P1319443).
-Investigate the effect of Ca on Sr and Cs accumulation.

In this future experiment several *Brassica* species (including commercial varieties of broccoli and cabbage) will be tested together with the three varieties of *Ph. accutifolius, Vicia villosa, Vicia sativa Vicia faba,* sugarbeet, and *Agrostis alba.* The Ca/Sr ratio in the nutrient solution will be adjusted to: 100:1, 100:2 (Johnson solution) , or 100:3 by keeping Sr concentration constant (20 µM) and changing $Ca(NO_3)_2$ concentration to: 2.0 mM, 4.0 mM and 6.0 mM.

4. Preliminary investigation of the forms of U taken up by plants.

In order to devise a suitable screening system for uranium accumulation, information is required on the form of U available to plants in soil. There appears to be no published solution culture experiments on U and very few controlled experiments in soil where uptake has been measured in plant tissue. Geochemical studies on the Fernald site suggest that soluble U is present in the pH 6.5 soil chiefly as anionic carbonate species. This is due to the addition of alkaline, carbonaceous remediation and road construction materials. A considerable portion of U is also present as insoluble particulate matter. Geochemcial modeling shows that carbonate complexes of U are very stable and provide potential for leaching of U from the environment. Phosphate complexes of U are also very stable and would be present where soils are fertilized with P to improve plant growth. It is not known which of these forms of U are taken up by plants. Free UO_2^{2+} ions are likely to be present in the soil solution only at pH less than 5.5. If this form were taken up by plants, considerable modification of the soil environment would be required for successful phytoremediation.

An experiment was designed to investigate which forms of U might be taken up by plants from nutrient solution. The limitation of commencing a screen before this stage is that it will not be possible to ascribe failure of any plants to accumulate U to either their intrinsic properties or to unavailability of U to the plants in the soil solution. A solution of depleted $^{235}UO_2(NO_3)_2$ was supplied to a half strength modified Johnson's solution at 10 μM total concentration. Peas (cv Sparkle) were chosen as the test plant after promising results reported by Dr. Jianwei Huang of DuPont at the recent University of Missouri Phytoremediation Conference showed that this species accumulated lead in shoots. As the most stable complexes of U are with P (to the extent that no free U is present in a typical nutrient solution), the initial experiment was carried out with plants which were pre-cultured in high P solution and transferred to solutions containing U, but no P. 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 the free ion, UO_2^{2+}. Aat pH 6.5 most U was present as hydroxide complexes and at pH 8 nearly all U was present at carbonate complexes. Plants were pre-cultured at the test pH values for 2 weeks in the absence of U and then transferred to U for 7 days. Plants were also continuously grown in the absence of U at the test pH values to provide a toxicity control. Observations of roots at harvest revealed that U is quite toxic to roots with similar symptoms to Al toxicity such as stunting of lateral root growth and blackening of existing roots. The effect was most pronounced at pH 6.5.Analyses of shoot U concentrations have been inconclusive with large variation between replicates in terms of uptake of U. Values were most uniform at pH 5.0 with uptake in the order of 4 μg/g of U. However, results for pH 6.5 and 8.0 ranged from slightly above background to 20 μg/g. It is possible that pH fluctuations, particularly at pH 8.0, during the experiment led to different speciation conditions in replicate pots. The experiment will be repeated using a more controlled carbon dioxide gas buffering system to ensure uniformity between replicates. Future experiments will probably employ a lower concentration of U and will be conducted in the presence of P, thus reducing the activity of free U in solution.