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Phytoremediation of Soils Contaminated with Heavy Metals and Radionuclides

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3) Executive Summary

The objective of this project was to set up the technical basis to establish a cost effective and efficient system of removal of heavy metals (HM) and nucleides from contaminated soils of Kazakhstan and allow a renewed introduction of sustainable agriculture into these areas.

In order to achieve this objective research was conducted by the teams in Sede Boqer and Astana, sometimes by local investigators and other times by mixed participation of both groups. Dr. Alikulov came to work to Sede Boqer during periods making use of local expertise and instrumentation that was not available in Astana.

- Work in Israel has started with two types of plant species: (a) crops such as barley, pea, tomato and maize and (b) Some well known HM hyperaccumulators like Typha and Phragmites. Work focused on the determination of agrotechnical methodologies and biochemical characterization of key enzymes involved in the production of phytochelatines in order to understand better the key metabolic systems that provide the plant with means to chelate and accumulate heavy metals.
- Most hyperaccumulators store the heavy metals taken up from the soil in their roots which makes more cumbersome the harvesting of the storing plant organs from the field. This is a problem of xylem loading or in more biochemical terms, the activity of heavy metal transporters at the xylem parenchyma of plant roots. Work was carried out to find such transporters, which were eventually found in some Kazak native desert plants. Work is under way at the moment to identify the HM-transporter and isolate its gene for eventual transfer to other plant species used in phytoremediation of contaminated soil and water
- Work in Kazakhstan has been carried out with *Amaranthus*, *Agropyron* and *Aeluropus* species assessing the capacity of this species to remove heavy metals from soils and their accumulation in roots and shoots. Several methods to enhance the HM uptake capacity by these species have been studied and interesting new apporaches to facilitate HM uptake by the plants. The Kazak investigators have also determine the changes of HM removal by Amaranthus during the life cycle of the plants to determine effective stages of growth for optimal removal of HM from the soil
- Work has been carried out in parallel at the physiological, biochemical and field levels with good integration among the different components, as evidenced in some of the results shown. **The results obtained suggest the necesity of a larger scale pilot project to optimize the efficiency of the selected species to remove HM from contaminated soils under field conditions, based on the lessons learned so far under laboratory and greenhouse conditions. This pilot stage should be an intermediate stage betwee lab/greenhouse research and large scale pytoremediation of contaminated soils.**

All the work carried out during project was in compliance with the stated objectives indicated in the grant proposal. Collaboration between the two groups has been excellent with the active participation of Dr. Vladimir Kuzovlev and of Dr. Zerekbay Alikulov. The research unit of Alikulov in Astana has been the pride of the academic euthorities of Gumilev University and show case of initiative, hard work and scientific interest can build practically from nothing into a source of gifted and well motivated young and independent scientists. The development of this self-sustaining group and the research tools at their disposal are an excellent example of what AID/CDR support can achieve in Central Asia and other parts of the world. The group is perfectly capable to day to define and solve numerous problems related to the development of advanced agriculture and management of natural resources.

Quite a number of papers by the principal investigators of this project are in preparation due to further data analysis and time consuming task of improving on preliminary drafts. Results of this work have been presented in several International Scientific Conferences and in National Scientific Conferences in Kazakhstan and other Central Asia countries, where they elicited great interest from people interested in phytoremediation.

4) Research Objectives:

Overall aim: Removal heavy metals and radionuclides from contaminated soils by the use of selected annual crops capable of taking up and immobilizing in the plant biomass large amounts of the polluting agents.

Specific objectives:

1. Preliminary selection of appropriate endemic and/or non-endemic (a rare and endangered) annual plant populations with heavy metal accumulation potential, with anatomical characteristics which facilitate harvesting.

2. Study of the biosynthesis of metal-chelators and transporters in roots and shoots of the selected plants, and determination of optimal metal accumulation during the plant life cycle.

3. Application of simple agrotechniques such as fertilization with nitrate, sulfate and Mo to enhance biomass production and the chelating capacity of the selected plants.

4. Study of additional chelating mechanisms in the plants and determination of optimal conditions for heavy metal influx, transport and compartmentation.

As could be expected from the limited funds and the time available to this project only the preliminary aspects of selecting suitable native plants and studying their characteristics as possible hyperaccumulators of heavy metal has been accomplished. This, however, has been very instructive and it can be said today that we have several native plant species that are very promising phytoremediators of soils and water contaminated with heavy metal and/or radionuclides. **The next stage should be a pilot field project to test the effectiveness of the selected and studied plant species on the actual removal of heavy metals from contaminated soils and the optimization of large scale use of these plants to reclaim abandoned soils and return them to fruitful agricultural production.**

5) Methods and Results

5A – Studies on the heavy metal hyperaccumulation capacity of Kazakhstan native plant species

Plants absorb lead and accumulation of this metal has been reported in roots, stems, leaves and root nodules, increasing with the metal level in the soil. Some plants are able to tolerate excess of lead by involving processes like exclusion, compartmentalization or synthesizing metal detoxifying peptides or organic acids. Furthermore the metal remains largely as a surface deposit or coating of plant organs. Coating lead of foliage may contain up to 100 times the level observed in unwashed leaf surfaces of the crop. Pubescent leaves have been coated with more lead than the smooth surfaced leaves due to regular wash off the metal by the rain water from the smooth leaf surface (Karaganov et al., 1995).

In higher plants the monocots have been studied in more detail than the dicots for their responses to heavy metals. However, new studies are needed to better understand the responses of the plants and mechanism of tolerance to the metals, or accumulation of them in several plant species, particularly in halophytes. Halophyte plants are widespread in the area of southeastern Kazakhstan. The capability of several plants including halophyte to extract heavy metals from industrially contaminated soils and an agricultural soil contaminated with metals from sewage sludge were studied.

One of the main sources of heavy metals pollution in Kazakhstan are industrial installations as well as nuclear and non-nuclear weapon testing grounds which release not only heavy metals but also highly toxic radionuclides. Waste water and dust from mining industries and weapon testing grounds containing heavy metals and radionuclides contaminated vast areas of Kazakhstan.

As part of numerous active efforts by science and technology to develop better and cost effective technologies to remedy contamination of soils of polluted areas, the value of plants as cleansers of environmental pollutants is becoming increasingly appreciated. Interest in phytoremediation has rapidly expanded in the last few years. The success of this endeavor is based on the capacity of plants to take up and accumulate heavy metals based on their uptake by roots, coupled to rapid plant growth and a simple harvest (Salt et al., 1995; Cunningham and Ow 1996). Plants absorb heavy metals that are accumulated in roots, stems, leaves and root nodules, increasingly with the increase in metal level. Some plants are able to tolerate excess of lead by involving processes like exclusion, compartmentalization or synthesizing metal detoxifying peptides or organic acids.

The content of lead, cadmium and zinc is generally higher in south-eastern Kazakhstan than reported in many studies of other contaminated regions of the world and they can exceed by many fold the maximal allowed concentrations. South-east Kazakhstan includes Oskemen, Kyzilorda and Shymkent districts.

In Kyzilorda which is located in the Aral Sea basin in Kazakhstan, the environmental problems are of catastrophic proportions. As a result of the implementation of a massive irrigation schemes to support the cotton and rice fields in the former desert virgin land, the water flow to the Aral Sea was reduced to less than half. As a result, not only its NaCl content but also that of heavy metals have increased in the water and soil and have been deposited over large areas by winds, entering the food chain ending in the large human population of cities.

Alarming concentrations of lead have been reported in dust of densely populated urban areas such as Shymkent. Water and land of this area near the industrial waste disposals. The main lead producing plant is located in this city in Kazakhstan dating from the Soviet period. The level of lead in the environment is not constant and has been increasing rapidly due to industrialization during the last several decades. An increase of total and available lead in the soils in Shymkent has been observed. Irrigation with untreated sewage effluents (Karaganov et al., 1995) increased soil contamination. Another major source may be lead alkyl derivatives in gasoline which are cracked to release lead in automobile exhaust in the form of lead aerosols (Smith, 1971). This results in extensive contamination of the metal near road side. The soil and the plants along the roads to Shymkent are rich in lead content. The metal tended to accumulate in the surface layer where at 0-15 cm depth in last three decades total lead increased from 8.5 mg/kg to 27.4 mg/kg, and detected a very high 320.5 mg/kg lead in street dust (Karaganov et al., 1995).

Plants absorb lead and accumulation of the metal has been observed in roots, stems, leaves and root nodules which increased with the increase in the exogenous metal level. Some plants are able to tolerate excess of lead by involving processes like exclusion, compartmentalization or synthesizing metal detoxifying peptides or organic acids. Furthermore, the metal remains largely as a surface deposit or topical aerosol coating plant shoots. Lead associated with foliage coating may contain up to 100 times more lead than the unexposed leaf surfaces of the crop. Hairy leaves have been coated with more lead than the smooth surfaced leaves due to the frequent rinse of the metal by the rain water from the smooth leaf surface (Karaganov et al., 1995).

In higher plants the monocots have been studied in some detail for their responses to heavy metals in general than the dicots. However, new studies are needed to better understand the responses of the plants and mechanism of tolerance to the metals, or accumulation of them in several plant species, particularly in halophytes. Halophyte plants are widespread in the area of south-eastern Kazakhstan. Therefore we studied the capability of several plants, including halophyte plants, to extract heavy metals from industrially contaminated soils and an agricultural soil contaminated with metals from sewage sludge.

Materials and Methods

Seeds of *Amaranthus* and *Agropyron* were obtained from the Institute of Botany and Plant Introduction of the Kazakh Academy of Sciences. Seeds of Aeluropus were collected from plants in sandy saline soils, in the Kazali region of the Aral Sea in September-October 2001. Soils for the studies were collected from Shymkent where a lead-extracting plant is located, and from Kyzilorda near the Aral Sea. Uncontaminated control soils were collected from the Turkistan region located between the cities of Shymkent and Kyzilorda.

Seed priming method. Dry dormant seeds of the plants were imbibed in distilled water or in water solutions of molybdate or tungstate. The seeds fully saturated with the water or with the solutions during 30 h imbibition. Then the seeds ere dried on filter paper at room temperature, the seeds reached their original dry weight after 25-30 h. Germination of the seeds were carried out on moist filter paper in Petri dishes or in sandy soil with or without heavy metal. Contact of the seeds with oxygen during imbibition and subsequent drying led to germination inhibition while the germination percent of the seeds imbibed under oxygen was high if the seeds germinated immediately after imbibition, i.e. omitting the drying step.

Foliar fertilization. Foliar applications were carried out during the early morning when the stomata were open and temperatures moderate.

Inoculation with vascular-arbuscular myccorhizae (VAM). VAM fungal inoculum was generated from *Aeluropus* plants collected from sandy saline soils in the Aral Sea area of Kazakhstan and, Agropyron collected from the steppe near Astana city. *Amaranth* species was collected from different regions of Kazakhstan had no VAM inoculum. To produce the VAM fungal inoculum, *Aeluropus litoralis* and *Agropyron desertorum* plants with intact mycorrhizal roots and adhering soil were transplanted into 15 cm diameter pots containing autoclaved contaminated soil collected from Shymkent (27.4 mg Pb^{2+}/kg soil), and uncontaminated soil collected in the Turkestan area. After growth during 1 month, the pot contents became the source of infective inoculum for our experiments (Kasymbekov et al., 2000). The dominant fungus present in the pots was identified as *Glomus clarocystis*.

Preparation of tissue extracts. Leaves and roots of the plants were macerated with acid washed sand in ice-cold extraction buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM GSH, 10 μM leupeptin, 0.01 mM Na_2MoO_4 and 0.01 mM FAD. All the extractions were done at a ratio of 100 mg fw/1 ml buffer. The homogenate was centrifuged at 30 000 g for 20 min at 5°C and the resulting supernatant was used for analysis.

Determination of MoCo activity. Cofactor activity in seed tissue was estimated using a NR-mutant nit-1 of Neurospora crassa. The mutant of N. crassa was grown in the Department of Biotechnology and Selection of the Plants (National Biotechnological Center in Astana). MoCo activity was determined using the highly sensitive modified method developed by Savidov et al. (1998).

Determination of enzyme activities. The activity of wheat NR in vitro was determined by using NADH and also reduced methyl viologen (MVH)), an artificial electron donor (Alikulov et al., 1988. *In vitro* XDH activity was determined according to Triplett at al (1982) following NADH production at 340 nm. AO activity was assayed monitoring the change of absorbance at 600 nm of the electron donor 2,6-dichloroindophenol (DCIP) (Koshiba et al., 1996). Absorbance of enzyme products was measured using a Jenway spectrophotometer. Before determination of the activities of AO and XDH, the extracts of seed parts were gel-filtered through Sephadex G-25 to separate low molecular compounds which may contain endogenous NADH and other reductants and may interfere with the cofactors and products of the enzyme reactions studied.

In our experiments $Cd(CH_3COO)_2 \cdot 2H_2O$ and $Pb(NO_3)_2$ were used as heavy metals containing compounds. The content of heavy metal was determined using the spectrometer AAS-IN (FRG) following mineralization of plant materials by inorganic acids (details in previous report).

Results and Discussion.

Efficient phytoextraction of heavy metals from the soil requires high plant biomass with efficient uptake systems. Halophytes characteristically accumulate large quantities of salts in shoots and can produce high biomass. Salt-tolerant plants may also be tolerant of heavy metals and capable to accumulate metals. In our study we compared metal uptake by the halophyte *Aeluropus litoralis* and the glycophyte *Agropyron desertorum*, and also a moderate salt-tolerant *Amaranthus tricolor*.

Table A-L. Toxicity thresholds of Pb^{2+} and Cd^{2+} for *Amaranthus, Agropyron* and *Aeluropus* (percent of survival in the heavy metals).*

Heavy metals	Concent. mM	Amaranthus	Agropyron	Aeluropus
Lead	0 (control)	100	100	100
Cadmium		100	100	100
Lead	0.1	100	100	100
Cadmium		100	100	95
Lead	0.5	100	100	100
Cadmium		93	95	84
Lead	1.0	95	93	97
Cadmium		92	86	70
Lead	1.5	85	65	96
Cadmium		60	57	55
Lead	2.0	94	40	75
Cadmium		42	23	34
Lead	2.5	90	15	55
Cadmium		17	2-5	20

**Plant seeds were germinated in sandy soil. After 10 d of seedlings growth different concentrations of heavy metal were applied to the soil and seedlings were further grown until their rate of survival (at 25 d) was estimated*

In order to provide a better estimate of plant toxicity thresholds to heavy metals we studied the tolerance of *Amaranthus, Agropyron* and *Aeluropus* exposed to various Pb^{2+} and Cd^{2+} levels. In our previous experiments we studied the effects of heavy metals such as Cd, Zn and Cu on the growth of *Amaranthus tricolor* and *A. paniculatus* and their metal-accumulating capability. In these experiments we also used Cd in order to compare its effects with those of Pb. A greenhouse screening study was carried out, in which the seedlings of these plant species were grown in sandy soil and exposed to different concentrations of soluble Pb^{2+} and Cd^{2+} salts ranging from 0 (control) to 2.5 mM. Cadmium was found to be more toxic for the plants tested than lead (Table A-1). The levels of endogenous metals increased with exogenous lead and cadmium levels. Furthermore, the toxicity threshold of Cd and Pb was found to be 1.0 mM for all the plant species studied.

Since the halophyte *Aeluropus* grows preferentially in saline soils, we tested the effects of increased soil salinity on heavy metal accumulation by these plants using as controls *Amaranthus* and *Agropyron*. Preliminary results showed that *Aeluropus litoralis* grew on soils with salinity up to 6.0% in the Aral Sea basin (Sarsenbaeva 2000). According to data published by the Institute of Botany and Phytointroduction of the Academy of Sciences of Kazakhstan, *Aeluropus litoralis* is one of the dominant plants in grazing lands of the Aral Sea basin. All these halophytes have a remarkable ability to accumulate large quantities of NaCl in their tissues. The mineral content of these plants, mainly NaCl, was between 30 and 50% of their dry weight (Jefferies and Rudnik 1964). The main salt accumulating organ of *Aeluropus* were found to be its leaves (Sarsenbeva 2000). Since *Aeluropus* is able to accumulate salts in its leaves, we tested the plant for heavy metal uptake and accumulation.

Table A-II. The effects of increasing NaCl concentrations on Pb^{2+} accumulation (mg/g DW) by different plant species (1.0 mM Pb^{2+} added to NaCl solutions)*.

Plant species	Plant parts	Concentrations of NaCl					
		0	1%	2%	3%	4%	5%
Amaranthus	Pb^{2+} in roots	18.7	13.3	6.0	IW	-	-
	Biomass	97	92	65	-		
	Pb^{2+} in leaves	17.0	12.7	4.8	IW	-	-
	Biomass	100	96	60	-		
Agropyron	Pb^{2+} in roots	14.5	10.3	4.6	IW	-	-
	Biomass	95	83	35	-		
	Pb^{2+} in leaves	18.0	12.3	6.5	IW	-	-
	Biomass	97	80	28	-		
Aeluropus	Pb^{2+} in roots	15.6	15.2	15.4	17.8	15.9	14.2
	Biomass	100	100	102	105	94	83
	Pb^{2+} in leaves	18.5	25.4	32.5	48.2	50.2	48.5
	Biomass	100	103	107	106	98	87

**Plant seeds were germinated in sandy soil. At 10 d of seedling development a solution with 1.0 mM Pb^{2+} and increasing concentrations of NaCl was supplied to the soil. Biomass was estimated in % of control seedlings grown without Pb and NaCl; IW – growth of seedlings was inhibited and wilted after 10 days in the presence of NaCl. Biomass and the content of lead in plant materials were measured in 35 d old plants.*

The monocots *Aeluropus* and *Agropyron* accumulated considerably more Pb in their leaves than in their roots. The halophyte translocated more Pb from the root to the shoot than the glycophyte, while amaranth accumulated almost equal amount of the metal in its roots and shoots. Our results suggest that the halophyte has a greater potential to phytoextract Pb from contaminated soils.

The accumulation of lead may depend not only on plant species but also on the exogenous concentration of lead in the environment. In the next experiments we tested the dependence of heavy metal accumulation on an exogenously supplied concentration of lead (Table A-III).

The effects of vesicular-arbuscular mycorrhizae (VAM) on lead accumulation by the plants were studied. Saprophytic fungi simultaneously colonize the utricle, the hard porous capsule of the seed testa and root cortex cells of the emerging radicles during germination, a colonization that affected positively seedling vigor (Barrow et al., 1997). Such symbiotic associations may improve salt tolerance of developing seedlings of *Aeluropus*. Although increasing concentrations of NaCl inhibit VAM growth, the hyphae placed onto the roots is not affected by NaCl (McMillan et al., 1998). Mycorrhizae colonized plants produced greater root and shoot dry weights than non-mycorrhizal controls (Ruiz-Lozano et al., 1996). Among the plants grown in native saline soils the maximum root VAM colonization was observed in *Aeluropus* species (Bhaskaran and Selvaraj 1997). Mycorrhizal fungi play a role in whole-plant nutrient balance by aiding in the uptake of limiting nutrients, maintaining nutrient balance under stress conditions (Ning and Cumming 2001).

Table A-III. Dependence of Pb^{2+}-accumulation in plant parts of various species on metal concentrations exogenously supplied to sandy soil (metal content in mg/g DW)

Plant species	Plant parts	Lead in medium (mM)	Pb in the plant mg/g DW
Amaranthus tricolor	Roots	0.0	1.8
		0.5	7.2
		1.0	18.5
	Leaves (+shoots)	0.0	1.0
		0.5	8.5
		1.0	15.7
Agropyron desertorum	Roots	0.0	1.7
		0.5	12.6
		1.0	15.0
	Leaves	0.0	2.3
		0.5	12.5
		1.0	17.5
Aeluropus litoralis	Roots	0.0	1.4
		0.5	9.5
		1.0	14.3
	Leaves	0.0	2.1
		0.5	13.0
		1.0	18.3

In the following experiments the effects of an inoculation of the plants with the fungus *Glomus* were studied. No infection of amaranth plants by the fungus was observed (Table A-IV) presents the results obtained from these experiments.

Table IV. Effects of plant colonization with fungus *Glomus* on lead accumulation in the roots and leaves of *Agropyron* and *Aeluropus.**

Pla[nt] species	Growth substrate	Lead accumulation (mg/g DW)			
		Roots		Leaves	
		Biomass	Pb^{2+}	Biomass	Pb^{2+}
Aeluropus	Uncontaminated soil	100	0.6	100	0.1
	Contaminated soil (CS)	100	5.1	100	5.6
	CS + NaCl	100	6.2	98	6.5
	CS + inoculum	108	9.3	106	12.8
	CS + NaCl + inoculum	109	12.2	107	19.7
Agropyron	Uncontaminated soil	100	0.4	100	0.2
	CS	97	4.7	100	7.4
	CS + inoculum	103	10.5	104	17.2

**Plant seeds were germinated in contaminated soil collected from Shymkent and in uncontaminated soil from the Turkestan area. Biomass and the content of lead were determined in 35 d old plants. After 10 d of seedling development, 3% NaCl was added to the soil.*

When colonized with vascular-arbuscular miccorhizae, *Agropyron* and *Aeluropus* roots and leaves accumulated higher levels of lead (Table A-IV). Moreover, the level of endogenous metal increased with exogenously supplied lead concentrations (not shown). However, attempts to inoculate amaranth plants were not successful. Moreover, no VAM inoculum was detected in the roots of *Amaranth* species from different regions of Kazakhstan.

Lead in the soil is present in the form of soluble and insoluble salts and is tightly bound to colloidal organic molecules (Karaganov, 1995). Plant roots studied are able to extract some of this metal from the soil but translocation to shoots is generally limited. Various soil conditions such as cation exchange capacity and other ions alter lead uptake from the soil. Abundant amounts of phosphate form insoluble precipitates of lead phosphates which reduce metal absorption (Singh et al., 1994). Thus, another requirement for efficient phytoextraction is that the metal contaminants be in a form available for plant uptake. Chemically enhanced phytoextraction by adding ethylenediaminetetraacetic acid (EDTA) is well documented. We studied the effect of EDTA on lead accumulation of root and shoots of growing seedlings (Table A-V).

Table A-V. Effects of increased concentrations of EDTA supplied to contaminated soil on lead accumulation by the plants*.

Plant species	EDTA supplied	Plant parts	Biomass % of control	Pb^{2+} in plant mg/g DW
Amaranthus	Without EDTA (control)	Roots	100	5.6
		Leaves	100	5.3
	1 mg/kg	Roots	100	7.8
		Leaves	100	8.0
	10 mg/kg	Roots	100	9.5
		Leaves	100	10.4
	100 mg/kg	Roots	100	11.5
		Leaves	100	15.2
	1 g/kg	Roots	94	19.4
		Leaves	98	26.0
	10 g/kg	Roots	78	19.2
		Leaves	85	55.7
Agropyron	Without EDTA (control)	Roots	100	3.5
		Leaves	100	6.0
	1 mg/kg	Roots	100	5.7
		Leaves	100	7.2
	10 mg/kg	Roots	100	8.4
		Leaves	100	10.7
	100 mg/kg	Roots	100	11.3
		Leaves	100	16.5
	1 g/kg	Roots	96	18.6
		Leaves	100	23.6
	10 g/kg	Roots	85	18.3
		Leaves	95	23.5
Aeluropus	Without EDTA (control)	Roots	100	4.2
		Leaves	100	6.0
	1 mg/kg	Roots	100	5.3
		Leaves	100	8.3
	10 mg/kg	Roots	100	11.4
		Leaves	100	13.2
	100 mg/kg	Roots	100	15.8
		Leaves	100	29.4
	1 g/kg	Roots	100	25.4
		Leaves	100	53.4
	10 g/kg	Roots	98	23.5
		Leaves	95	52.5

**Plant seeds were germinated in contaminated soil collected from Shymkent, adding to the soil increasing concentrations of EDTA as indicated in the table. Biomass and the content of lead measured in 35 d old plants were determined.*

Thus, chelator application strongly increases plant lead uptake from metal-contaminated soils. As shown in Table A-V, EDTA in higher concentrations can be toxic for the plant. EDTA is nonbiodegradable in nature and very costly for large-scale phytoremediation, limiting its usefulness for continued soil application. In this respect, one of the most prospective and promising directions in phytoremediation is the use of biohumus. **Biohumus** contains a number of ingredients some of which chelate heavy metals. It may be applied at approximately similar rates of application as conventional chemical fertilizers. Experiments on the use of biohumus for phytoremediation are reported bellow.

Study on physiological and biochemical mechanisms of lead effects

Seed germination and early seedling stands are the initial events in the life of a plant projecting the extent of future physiological and biochemical processes. Seed germination is initiated by regulated enzyme reaction which activates catabolic and anabolic processes in the storage tissue (cotyledons and endosperm) and in the embryonic axis. Germination is inhibited even when only one of these component processes is affected.

Toxic levels of Pb affect plant development at physiological and biochemical levels. As the metal reacts with important functional groups in macromolecules, the activities of several enzymes are affected, some of which are important in plant adaptation to unfavorable environmental conditions and nitrogen metabolism. The inhibition of germination by exogenously lead may affect some important enzymes involved in the process. The inhibitory effect of lead on seedling growth may arise from interference of lead with auxin (indole acetic acid, IAA) regulated cell elongation (Van Assche and Clijsters, 1990). Molybdenum containing enzymes are inhibited in roots of monocots by the metal but in the leaves a differential effects are observed in monocot species and dicots as well (publication in preparation).

An oxidase catalyzing the oxidation of indole acetaldehyde (IAAld) and abscisic aldehyde (ABAld) to indole acetic acid (IAA) and abscisic acid (ABA), respectively, is found to be the molybdenum containing enzyme aldehyde oxidase (Koshiba et al., 1996). Much attention has recently focused on plant aldehyde oxidase (AO, EC 1.2.3.1) because of its involvement in the adaptation processes of plants to the environmental stresses (Omarov et al., 1998). It is proposed that the main function of plant XDH is the production of uric acid which plays an important role in the scavenging of ROS formed during the oxidative stress.

Nitrate assimilation is widespread among plants. The regulating step of this process appears to be the initial reaction catalyzed by NR. This enzyme is considered to be a limiting factor for growth, development, protein production and final yield of plants (Hageman and Lambert, 1988). Nitrate assimilation is inhibited by 10 to 100 mM Pb^{2+} in different species of higher plants studied. Relatively lower concentration 100 µM of lead inhibited nitrate uptake and *in vivo* NR activity in cucumber seedlings (Burzynski and Grabowaski, 1984). The activity of NR is also inhibited in soybean (Huang et al., 1974), maize and pea leaves (Sinha et al., 1988).

Lead treatment of seedlings can create water stress to the plants (Burzynski and Grabowski, 1984), i.e. it causes the oxidative stress in plant cells. Formation of reactive oxygen species (ROS, highly reactive oxygen radicals) is a common reaction at the initial steps of several types of stress including exposure to heavy metals.

The effect of Pb^{2+} on root MR enzyme seems to be due primarily to its interference with the *de novo* synthesis of NR molecules. Induction of NR activity was significantly inhibited by Pb^{2+} (0.5 mM) in the *Agropyron* leaves. Among various heavy metals tested, Zn^{2+} and Cu^{2+} to some extent counteracted the inhibitory effect of Pb^{2+} on both *in vivo* and *in vitro* NR activities (data not shown).

The toxicity of Pb^{2+} on Mo-enzyme activities could be ameliorated by MoO^{4-}. The response of NR activity to lead is different when the enzyme is assayed *in vivo* or *in vitro* methods, whereas the *in vivo* NR activity was generally inhibited (Table A-VI), the *in vitro* assay was largely unaffected (data not shown).

Table A-VI. Effect of 1.0 mM Pb^{2+} supplied to sandy soil on the activities of plant Mo-enzymes*.

Plant species	Plant parts	MoCo		AO		XDH		NR	
		Control	+Pb	Control	+Pb	Control	+Pb	Control	+Pb
Amaranth	Roots	1.8	1.4	43.0	39.5	1.2	1.0	2.7	2.0
	Leaves	2.1	1.6	70.5	60.3	2.9	2.6	5.0	3.8
Agropyron	Roots	2.4	1.8	67.4	61.0	2.5	2.2	5.4	4.2
	Leaves	1.8	1.5	40.3	38.5	1.6	1.4	3.7	3.2
Aeluropus	Roots	3.0	2.4	80.6	75.8	3.4	3.2	5.6	4.5
	Leaves	2.4	1.6	66.2	62.7	2.1	1.8	2.7	2.0

*<u>Activities:</u> *MoCo in µmol NO_2 formed by nit-1 NR mg^{-1} protein $hour^{-1}$,*
AO in nmol DCIP mg^{-1} protein min^{-1}, XDH in µmol NADH mg^{-1} $hour^{-1}$,
NR in µmol NO^{-3} mg^{-1} protein $hour^{-1}$

A possible direct effect of heavy metal on the enzyme may be due to the metal high binding affinity to SH groups. In the absence of molybdenum, newly synthesized MoCo could be inactivated by heavy metals such as Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+} and arsenite (Wahl et al., 1984; Rajagopalan, 1992). These metals show higher affinity to the vicinal dithiols than to monothiols (Simons et al., 1990; Aposhian et al., 1993; Ercal et al., 1996). Incubation of dithiols with Cd-saturated metallothionein (MT, a polypeptide containing regular monothiols) resulted in removal of the metal from MT showing the high chelating capability of dithiols (Zheng et al., 1990). We showed also that 2,3-dimercaptopropane-1-sulfonate was able to remove heavy metals bound to MoCo activating the cofactor.

The side chain of MoCo contains vicinal dithiol groups (Rajagopalan, 1992). Our experiments with active MoCo isolated from wheat embryo showed that heavy metals such as Hg^{2+}, Pb^{2+} and Cd^{2+} have high affinity to MoCo dithiols. The presence of relative high concentrations of MoO^{2-} and reduced condition protected MoCo dithiols against heavy metal binding (manuscript in preparation). It seems that Mo-deficiency and the presence of the heavy metals lead to the inactivation of newly synthesized MoCo by the latter in *in vivo* assays.

To test these hypotheses we used seed priming with molybdate and its chemical analog tungstate. Imbibition of the seeds in molybdate solution up to 50 mM did not inhibit their germination. In a separate experiment we studied the infiltration of 50 mM ammonium molybdate into the seeds during 30 h imbibition. The content of ammonium ions in the seeds determined with Nessler reagent was relatively high. The effects of seed priming with molybdate (or tungstate) on the activities of Mo-enzymes in plant seedlings grown in the presence of Pb was studied (Table A-VII).

Seed priming with molybdate resulted in a considerably increase of plant biomass (Table A-VII). Since efficient phytoextraction of heavy metals requires high biomass production of plants, seed priming of metal-accumulating plants may have a positive effect in phytoremediation efficiency. Seed priming also improves seedling stand size resulting in higher yield, and may be therefore expected that the plants grown from Mo-primed seeds will have a higher Pb-accumulating capacity.

Table A-VII. Effects of seed priming on the activities of Mo-enzymes in 5 days old seedlings.

Seed priming conditions	Plant parts	Biomass	MoCo	AO	XDH	NR
Amaranthus tricolor						
Control (dry seeds)	Roots	100	1.6	43.5	1.4	3.0
	Leaves	100	2.3	68.6	2.9	5.4
Control (dry seeds), grown in presence of Pb^{2+}	Roots	96	1.3	39.0	1.2	2.2
	Leaves	100	1.6	61.6	2.6	3.4
Seeds + priming in H_2O	Roots	**106**	**1.8**	**46.4**	**1.7**	**3.3**
	Leaves	**109**	**2.6**	**70.2**	**2.8**	**5.7**
Seeds + priming in H_2O, Grown in presence of Pb^{2+}	Roots	100	1.3	40.2	1.4	2.4
	Leaves	103	1.5	63.0	3.0	3.6
Seeds + priming in Pb^{2+}, grown in the water	Roots	83	0.6	18.7	1.0	1.2
	Leaves	86	0.4	37.5	2.7	1.2
Seeds + priming in Pb^{2+} grown in presence of Pb^{2+}	Roots	45	0.4	13.7	0.7	0.5
	Leaves	40	0.3	23.6	1.8	0.4
Seeds + priming in MoO^{4-}	Roots	**111**	**2.1**	**48.8**	**1.9**	**3.6**
	Leaves	**112**	**2.8**	**73.0**	**2.9**	**6.2**
Seeds + priming in MoO^{4-}, grown in presence of Pb^{2+}	Roots	107	2.2	47.8	1.9	3.5
	Leaves	106	2.8	72.5	2.8	6.0
Seeds + priming in WO^{4-}	Roots	100	1.7	17.0	1.5	1.8
	Leaves	100	2.4	23.0	1.9	1.2
Seeds + priming in WO^{4-}, grown in presence of Pb^{2+}	Roots	57	1.2	0.9	1.2	0.4
	Leaves	55	2.0	0.4	0.8	0.0

Agropyron desertorum						
Control (dry seeds)	Roots	100	2.5	68.0	2.4	5.0
	Leaves	100	1.8	41.0	1.8	3.6
Control (dry seeds), grown in presence of Pb^{2+}	Roots	95	1.7	60.5	2.2	4.0
	Leaves	92	1.3	36.0	1.6	3.0
Seeds + priming in H_2O, grown in the water	Roots	**110**	**2.8**	**70.2**	**2.4**	**5.2**
	Leaves	**107**	**2.0**	**43.3**	**1.9**	**3.5**
Seeds + priming in H_2O, grown in presence of Pb^{2+}	Roots	97	2.9	68.2	2.3	4.8
	Leaves	94	2.2	42.0	2.0	3.3
Seeds + priming in Pb^{2+}, grown in the water	Roots	85	2.6	62.3	2.0	3.8
	Leaves	82	2.2	36.6	1.6	2.7
Seeds + priming in Pb^{2+}, grown in presence of Pb^{2+}	Roots	48	1.8	39.4	1.5	1.8
	Leaves	52	1.5	20.5	1.0	0.8
Seeds + priming in MoO^{4-}, grown in the water	Roots	**113**	**3.2**	**73.4**	**2.6**	**5.8**
	Leaves	**109**	**2.3**	**45.7**	**2.1**	**3.6**
Seeds + priming in MoO^{4-}, grown in presence of Pb^{2+}	Roots	105	3.3	72.7	2.6	5.3
	Leaves	108	2.2	46.3	2.3	3.2
Seeds + priming in WO^{4-}	Roots	97	2.9	28.4	1.8	1.9
	Leaves	100	1.2	19.0	1.2	1.4
Seeds + priming in WO^{4-}, grown in presence of Pb^{2+}	Roots	53	1.7	20.5	1.3	0.8
	Leaves	57	1.3	13.5	0.8	0.4
Aeluropus litoralis						
Control (dry seeds)	Roots	100	3.2	81.0	3.2	5.4
	Leaves	100	2.5	65.7	2.5	2.7
Control (dry seeds), grown in presence of Pb^{2+}	Roots	100	2.6	75.0	3.4	4.4
	Leaves	100	1.6	63.0	2.3	1.8
Seeds + priming in H_2O, grown in the water	Roots	**105**	**3.5**	**82.3**	**3.1**	**4.8**
	Leaves	**115**	**2.5**	**66.3**	**2.7**	**3.0**
Seeds + priming in H_2O, grown in presence of Pb^{2+}	Roots	103	3.5	78.8	2.8	3.5
	Leaves	107	2.5	64.0	2.6	2.3
Seeds + priming in Pb^{2+}, grown in the water	Roots	100	3.2	75.2	2.8	2.5
	Leaves	100	2.3	65.0	2.5	2.3
Seeds + priming in Pb^{2+}, grown in presence of Pb^{2+}	Roots	90	2.2	52.0	1.8	0.9
	Leaves	95	1.6	53.0	1.5	1.0
Seeds + priming in MoO^{4-}	Roots	**110**	**3.7**	**85.0**	**3.6**	**6.0**
	Leaves	**118**	**2.8**	**67.8**	**2.6**	**3.2**
Seeds + priming in MoO^{4-}, grown in presence of Pb^{2+}	Roots	108	3.6	80.6	3.0	5.4
	Leaves	112	2.5	64.4	2.8	2.9
Seeds + priming in WO^{4-}	Roots	100	3.3	30.2	2.0	0.6
	Leaves	100	2.2	27.3	1.2	0.3
Seeds + priming in WO^{4-}, grown in presence of Pb^{2+}	Roots	55	2.0	21.3	1.5	0.2
	Leaves	62	1.6	18.8	0.8	0.2

Thus, Mo-deficiency causes a decrease of the activities of Mo-enzymes, particularly of AO that plays an important role in the tolerance of the plants to environmental stresses. The investigations of Saicheva and Vunkova suggest Mo is very effective tp increase cold resistance and productivity of winter wheat grown on acid soil and snow cover (Vunkova et al., 1989), a phenomenon whose mechanism is still unclear. Heavy metals are very mobile and easily taken up by germinating seeds and growing seedlings in acidic soils. On the contrary Mo becomes less available with decreasing soil pH. Under these conditions, AO is inhibited by heavy metals, but treatment of seeds with Mo protects against heavy metal inactivation.

Plants have evolved a capacity to take up Mo via active transport systems in roots. The form of Mo in neutral and alkaline soils is the soluble anionic molybdate ion. This tetrahedral oxyanion resembles sulfate and phosphate in physico-chemical properties and, indeed, the Mo transport system bears much in common with the sulfate and phosphate transport (Heuwinkel et al., 1992). The contents of S and Mo in the plants are 0.1% and 0.00001%, respectively. Competition for uptake between S and Mo takes place in the presence of high SO^{4-} concentration at the root surface. In saline soils in the Aral Sea region 23% of total soluble salts is sulphate, and 50% is NaCl. In Kyzilorda the proportion of this ions is only 14% and 24%, respectively (Akhanov et al., 1996). At the same time, Mo content in the soils is below its critical concentration. These factors may factors that determine Mo-deficiency in plants, particularly in the Aral Sea region and Kyzilorda. Sulfur assimilation is important for phytochelatine synthesis (Heiss et al., 1999). Sulfur taken up from the soil increases the formation of metal-chelators such as phytochelatins and metallothioneins in metal-accumulating plants. Saturation of seeds with Mo through priming allows to escape the inhibition of Mo-uptake in radicles and roots by soil sulfate. Seed priming with molybdate increased considerable lead-accumulation by plants of Pb-contaminated and relatively sulfate-rich soil (Table A-VIII).

Table A-VIII. Effect of seed priming with molybdate on metal accumulation by plants grown from primed seeds*.

Plant species	Plant parts	Pb^{2+} accumulated in the seedlings (mg/g DW)	
		Plants grown from seeds primed in H_2O	Plants grown from seeds primed with MoO^{4-}
Amaranthus	Roots	7.4	8.7
	Leaves	8.0	9.7
Agropyron	Roots	4.4	5.4
	Leaves	6.5	7.3
Aeluropus	Roots	5.2	6.5
	Leaves	6.7	7.6

**Primed seeds were germinated in contaminated soil collected from Shymkent. Lead content was determined in 35 d old plants.*

Another possible way to escape the uptake barriers (soil pH, competition, etc.) by roots is foliar fertilization with these elements. Foliar fertilizer provides nutrition to the cells in the leaf when the demand in the plant is greater than uptake, or when there is a competitive uptake of the ions. Foliar fertilization enables to correct mineral deficiencies. The efficiency of foliar fertilizers is greater than soil applied fertilizers. One advantage of foliar nutrition is that it often brings about immediate improvement (within hours) in plant health and growth. Root Mo content was 8 times greater, after foliar application, than that in legume plants not receiving a foliar treatment of molybdenum (Bronick and Giller, 1991). The effects of foliar fertilization of molybdate on Mo-enzyme activities in plant parts and their Pb^{2+}-accumulating capacity were studied (Table A-IX)

Table A-IX. Effect of foliar fertilization with molybdate on Mo-enzyme activities and metal accumulation in the parts of different plant species*.

Plant species	Plant parts	AO		XDH		NR		Pb^{2+} accumulated	
		Cont.	FF	Cont.	FF	Cont.	FF	Cont.	FF
Amaranthus	Roots	40.2	42.2	1.2	1.3	1.8	1.8	18.0	17.7
	Leaves	58.7	61.5	2.5	2.5	3.5	4.6	15.3	15.6
Agropyron	Roots	61.5	63.8	2.3	2.4	4.2	4.4	14.8	15.0
	Leaves	37.6	40.2	1.3	1.2	3.0	3.8	17.0	16.5
Aeluropus	Roots	74.7	76.2	3.2	3.4	4.2	4.2	14.2	15.0
	Leaves	63.0	68.6	1.7	1.8	1.8	2.5	19.0	20.3

**Cont. – control plants without foliar fertilization; FF – foliar fertilized plants. Plants were grown in sandy soil containing 1.0 mM Pb^{2+} and from 10 d of seedling growth the leaves were sprayed every 3 days with 10 mM molybdate solution. Enzyme activities and lead content were determined in 35 d old plants.*

Foliar supplied Mo increased the activities of leaf Mo-enzymes but plant uptake of Pb did not increase accordingly. However, unlike seed priming foliar fertilization of the plants with molybdate did not increase the biomass of plant parts, but only leaf biomass of amaranth slightly increased at 35 days after fertilization (data not shown).

The results of these experiments and those that will be described bellow suggest innovative ways to increase a well designed growing methodology of plant species for phytoremediation, enhancing the uptake of contaminants beyond the natural capacity of the species used. This will be an important consideration to optimize the cleansing of contaminated soils and reclaim them for productive agricultural use.

5.B Copper Uptake and Effects on Seed Germination and Plant Growth in *Agropyron desertorium* and *Aeluropus litoralis*

Copper soil content is higher in Central than in most other contaminated regions of Kazakhstan. Alarming concentrations of copper have been reported in dust of densely populated urban areas such as Balkhash in its water and soils near the industrial waste disposals in this area.. The Soviets developed the main copper producing plant is located in this city in Kazakhstan. The level of copper in the environment has been increasing rapidly due to rapid industrialization during the last several decades.

Materials and Methods

Uniform seeds of *Agropyron desertorium* and *Aeluropus litoralis* were immerged in concentrated ethanol for 25-30 min to prevent bacterial growth, then washed with deionized water, and soaked in water for 12 h. The soaked seeds were placed in plastic dishes of 10 cm diameter with a layer of filter paper on the bottom. In each dish 10 seeds were evenly placed on the surface of the filter paper, and 10 ml analytical grade $Cu(NO_3)_2$ aqueous solution with certain Cu^{2+} concentration was added. The Cu^{2+} concentrations in the solutions were as follows: 0, 125, 250, 500 and 1000 μg ml^{-1}. Each treatment had three replicates. Exposure lasted 94 h (4 days) under dark condition at 25°C. Then the germination rate, root and shoot length were recorded.

A pot study was conducted to determine copper bioaccumulation and tolerance of the plants under controlled conditions. Uniform seeds were immerged in ethanol for 30 min, washed with deionized water and soaked in water for 12 h. The treated seeds were then sown in 180 g (DW) of acid-washed 3:2 (w/w) mixture of fine sand and vermiculite placed in round plastic pots of 20 cm diameter, each pot with 20 seeds. The pots were watered daily until the seeds germinated. The young seedlings were watered daily with full-strength Hoagand's solution for 4 weeks. Uniform seedlings (8) were retained in each pot and the others eliminated, the pots were randomly divided into 5 groups (control and treatments), each group with 3 replicates. Aqueous solutions of chemical grade $Cu(NO_3)_2$ with different Cu^{2+} concentrations were added to the growth media at the beginning of the treatment period. Cu^{2+} concentrations in the growth media were 0, 125, 250, 500 and 1000 μg g^{-1} (DW) for the control and the treatments respectively. The seedlings were placed in a greenhouse with 14 h light, 25/18°C day/night temperature. Excess nutrient medium leached out from the pots was collected in plastic saucers placed below each pot and returned to the pot. The plants were harvested 2 weeks after beginning of the copper treatment. Roots were carefully washed with tap water to get rid of the sand, and then washed with deionized water. Roots, stems and leaves of plants were separated and dried in an oven at 85°C for 2 days at which time dry weight was determined.

Heavy metals were extracted from the plant material and soil the by the method of dry and humid mineralization. Dried samples was ground and digested in concentrated HNO_3 for 14 h, of which the initial 12 h were at room temperature followed by 2 h at 60°C, then further digested with concentrated HNO_3-$HClO_4$ (3:2, v/v) for 3 h at 140-160°C. After cooling, the extract was diluted with 1 *N HCl* and made up to 25 ml. Reagent blanks were prepared by carrying out the whole extraction procedure but in the absent of sample. Content of heavy metals was determined by using the atomic-absorption spectrophotometer AAS-IN (Karl Zeiss, FRG).

Results and Discussion

The effects of various concentrations of copper on seed germination, root and shoot length of *Agropyron* and *Aeluropus* are shown in Table B-1 and B-2. Significant concentration-dependent inhibitions of seed germination as well as root and shoot length were observed. However, the response of seed germination, root and shoot length to Cu-treatment varied.

Table B-1. Seed germination, root and shoot length of *Agropyron desertorium* after 4 days of copper treatment.

Cu treatment ($\mu g\ ml^{-1}$)	Germination rate (%)	Root length (mm)	Shoot length (mm)
0	100.0 ± 0.00	12.8 ± 4.32	9.12 ± 4.02
125	100.0 ± 0.00	10.37 ± 4.43	8.13 ± 3.67
250	75.7 ± 6.76	7.56 ± 1.53	5.35 ± 0.93
500	66.3 ± 5.65	6.23 ± 0.86	3.20 ± 1.00
1000	47.5 ± 6.63	3.27 ± 0.73	1.52 ± 0.97

Results are means ± SD.

Table B-2. Seed germination, root and shoot length of *Aeluropus littoralis* after 4 days of copper treatment.

Cu treatment ($\mu g\ ml^{-1}$)	Germination rate (%)	Root length (mm)	Shoot length (mm)
0	100.00 ± 0.00	15.65 ± 11.20	13.16 ± 5.62
125	100.00 ± 0.00	13.35 ± 4.53	12.73 ± 3.63
250	96.67 ± 8.57	11.63 ± 5.53	11.45 ± 3.94
500	84.57 ± 7.86	9.26 ± 3.86	9.20 ± 3.50
1000	73.50 ± 7.77	7.47 ± 2.33	7.82 ± 3.87

Results are means ± SD.

In both plant species the germination rate in 125 $\mu g\ ml^{-1}$ is the same than the control. Even at the highest Cu concentration (1000 $\mu g\ ml^{-1}$), *Agropyron* germination was still 48% of the control. While for *Aeluropus* it was 74% of the control. This is not the case for root length. The root length of seedlings in 125 $\mu g\ ml^{-1}$ Cu was slightly different from the control while in plants kept in 1000 $\mu g\ ml^{-1}$ Cu, the root length was only 25% of the control root. According to the length difference of the root and shoot of *Agropyron* between the lowest Cu concentration (125 $\mu g\ ml^{-1}$) and the control, highly significantly vs. non-significantly, the root seems to be more sensitive to copper than the shoot. *Agropyron* roots sensitivity to toxic metals may be due to the fact that roots were the specialized uptake organs and were affected earlier and subjected to accumulation of more heavy metals than any of the other organs. This could also be the main reason that root length was usually used as a measure for determining heavy metal-tolerant ability of plant.

Plant biomass of *Agropyron* and *Aeluropus*, after two-weeks culture in pots with various concentrations of copper are presented in Table 3 and 4. The mean biomass after two-weeks culture in the Cu-supplied medium correlates with seed germination and root and shoot length of *Agropyron* (Table 1), is also significantly different from the control as well as from each other (Table B-3)

Table B-3. Biomass (DW) of *Agropyron desertorium* after two-weeks of culture in Cu-enriched medium.

Cu ($\mu g\ g^{-1}$)	Root (mg)	Shoot (mg)	Total (mg)
0	8.6±2.5	20.0±5.2	28.6±7.7
125	7.0±2.3	18.3±4.2	25.3±6.5
250	6.7±0.6	18.0±2.2	24.7±3.0
500	5.0±2.6	14.0±1.7	19.0±4.3
1000	3.0±1.0	8.7±3.6	11.7±4.6

Results are means ± SD.

It is interesting to note that the biomass of *Aeluropus* shows a increasing instead of decreasing tendency as was expected due to increasing of copper concentration in the growth media (Table B-4). The response pattern of *Aeluropus litoralis* to the supplied Cu^{+2}, according to the biomass, seems to be a combination of some stimulating and inhibiting effects, presumably due to the combination of Cu and NO_3^-. The biomass of *Aeluropus* reaches its maximum at 500 µg g^{-1} Cu, and then declines at 1000 µg g^{-1}. This might suggest that the stimulating effect to the plant growth had been of dominance over the inhibiting before the copper concentration in the growth media increased to 1000 µg g^{-1}, and thereafter the inhibiting effect became stronger.

Table B-4. Biomass (DW) of *Aeluropus litoralis* after two-weeks of culture in Cu-enriched medium.

Cu concentration (µg g^{-1})	Root (mg)	Shoot (mg)	Total (mg)
0	9.3±1.5	18.0±6.2	27.3±7.8
125	9.0±2.0	21.3±3.2	30.3±5.0
250	9.7±0.6	25.3±4.0	36.0±4.4
500	13.0±2.6	30.0±7.7	43.0±9.3
1000	9.0±1.7	25.7±8.6	34.7±10.2

Results are means ± SD.

Regardless of the mechanism involved in the stimulation of plant growth in the presence of $Cu(NO_3)_2$ in the growth media, *Aeluropus* could be considered as a Cu-tolerant species, since even at the highest treatment (1000 µg g^{-1}), the biomass is higher than that of the control (no $Cu(NO_3)_2$ added)

The copper concentrations in the roots, stems and leaves of *Agropyron* and *Aeluropus* grown in sand/vermiculite mixture supplemented with Cu $(NO_3)_2$ are listed in Table 5 and 6. Copper concentration in the plant is significantly affected by both the copper content supplied in the growth medium and different plant organs of *Agropyron* as well as by the interaction between these two factors (Table 5). In all the organs of *Agropyron* the mean Cu concentrations increase without exception as the metal contents in the growth medium increase. The copper concentration in the root is, to a large extent, directly proportional to the metal content in the growth medium. When the copper supplied in the growth medium doubles, the metal accumulated in the root of *Agropyron* also doubles as compared to the copper concentrations in the root between any pair of the vicinal treatments (Table B-5). However, no such double-increasing pattern of copper concentration is observed in the cases of stem and leaf.

Table B-5. Copper concentrations in root, stem and leaf of *Agropyron desertorium* after two-weeks of culture in Cu-enriched medium.

Cu (µg g^{-1})	Root (µg g^{-1})	Stem (µg g^{-1})	Leaf (µg g^{-1})
0	125±57	31±20	26±13
125	1065±211	44±30	65±14
250	2215±378	145±67	466±97
500	4680±512	575±112	1320±172
1000	9275±1024	1287±276	2670±537
% accum	70.1	9.7	20.2

Results are means ± SD

The heavy metal content of plant tissue is important in determining their sites of accumulation in the plant. Numerous studies demonstrate that heavy metal concentration in plant is a function of heavy metal content in the environment. When the soil is heavily contaminated, the plants take up the metal through their roots (Nwosu et al. 1995; Xiong 1997). In the case of *Agropyron*, the plant roots are the largest accumulators of copper contamination (2/3), while the stems and leaves accumulate about 1/3 of the copper taken up. Thus the copper concentration in the roots of *Agropyron* is more closely related to that of the growth medium than in the stems and leaves.

Table B-6. Copper concentrations in root, stem and leaf of *Aeluropus litoralis* after two-weeks of culture in Cu-enriched medium.

Cu ($\mu g\ g^{-1}$)	Root ($\mu g\ g^{-1}$)	Stem ($\mu g\ g^{-1}$)	Leaf ($\mu g\ g^{-1}$)
0	98±22	25±10	226±74
125	110±35	30±8	755±140
250	178±54	55±17	8468±1070
500	225±80	145±33	21228±1520
1000	317±110	573±122	42670±6374
% accum	0.7	1.3	98.0

Results are means ± SD

Aeluropus turned out to be a much more effective accumulator than Agropyron both in amount accumulated and preferred accumulation site. The use of Aeluropus would allow effective removal of Cu from the soil just by removal of the aerial part of the plant, leaving less than 1% of the Cu accumulated by the plant in the roots in the soil.

The crucial factors determining Cu distribution in different plant organs may lie in the Cu translocation process from roots to leaves. Several anatomical biochemical and physiological mechanisms may be involved in heavy metal translocation in the halophyte *Aeluropus litoralis*, storing most of the metal accumulated in aboveground organs in the case of soil contamination. Thus, the *Aeluropus* plant accumulated an unusually high level of copper in their tissues during the growth period under the experimental conditions described (Table 6). This means that the plants can be compared to transpiration driven pumps which can extract and concentrate copper from the growth medium. Theoretically, the higher the ratio of heavy metal content in plant to that in growth medium is, the more powerful the transpiration-pump will be. In our study, *Aeluropus* demonstrates a relatively powerful species in extraction of copper via root system, especially at the highest treatment (1000 $\mu g\ g^{-1}$). As far as the Cu-tolerant ability concerned, *Aeluropus* would also seem to be superior to Cu-hyperaccumulators, since it did not show inhibition symptoms during the Cu treatment, and its biomass was higher than that of the control even at the highest treatment (1000 $\mu g\ g^{-1}$) (Table 4), presumably to more efficient use of NO_3^- the accompanying anion of the Cu cation. Furthermore, according to the increasing tendency of copper concentration in *Aeluropus* leaves from 500 $\mu g\ g^{-1}$ to 1000 $\mu g\ g^{-1}$ treatment (Table 6), the plant ability to accumulate copper would not seem to have reached its peak at the 1000 $\mu g\ g^{-1}$ treatment. Higher copper concentrations in the plant would seem to be expected when the Cu content in the growth medium would be further increased.

Efficient phytoextraction benefits from higher biomass production with good translocation from roots to leaves. metal uptake by a halophyte *Aeluropus litoralis* and a glycophyte *Agropyron desertorum* was compared in this study. The levels of Cu increase equally both in roots and leaves of *Aeluropus* and *Agropyron* but copper concentrations were much higher in leaves than roots in *Aeluropus* suggesting a very effective xylem loading mechanism of Cu in the roots of *Aeluropus*, presumably due to the existence of a Cu transporter in xylem parenchyma. **This as yet hypothetical Cu translocator would be an important gene to isolate for eventual improvement of other heavy metal hyperaccumulators.** The transporter mechanism produces very high Cu concentrations in its leaves. *Aeluropus* is a good hyper-accumulator of NaCl in its leaves, a mechanism characteristic of halophyte plants in above ground tissue material while exhibiting a high biomass production. Salt-tolerant plants may also be heavy metal-tolerant and further may be able to accumulate metals compounds.

A higher shoot/root ratio of heavy metal content in *Aeluropus litoralis* is important in practical phytoremediation of heavy metal-contaminated soils. In our study, *Aeluropus litoralis* shows a 136 shoot/root ratio of copper content. It demonstrates a superior ability of *Aeluropus litoralis* to accumulate not only copper but also other heavy metals (see our previous reports) from the soil via the root to its shoot. This property can enable phytoremediation of the heavy metal-contaminated soils only by harvesting the thus simplifying the agricultural practices for soil decontamination of salt in general and heavy metals in particular. Furthermore, the cutting of the aboveground parts of *Aeluropus litoralis* allows intensive regrowth of new shoots, resulting in the increase of density and growth rate of the plant shoots resulting in enhanced heavy metal accumulation. Thus, our results **show that Aeluropus, an endemic Kazakhstan halophyte, is an excellent hyper-accumulator of heavy metals.**

Effect of biohumus on Cu accumulation by higher plants

For the successful practical application of phytoremediation a number of variables must be considered. Among the most important of these is the biomass and metal content of the plant material.

Heavy metals in the soil are present in the form of soluble or insoluble salts tightly bound to colloidal organic molecules (Karaganov, 1995). Plant roots of different species were able to extract some of the complexed metal from the soil and accumulated in root, although xylem loading and translocation to the aerial parts is generally limited. Various soil conditions such as cation exchange capacity and other ions alter heavy metal uptake from the soil. Thus, another requirement for efficient phytoextraction is that the metal contaminants can be in a form available for plant uptake. Chemically enhanced phytoextraction using the treatment with ethylenediaminetetraacetic acid (EDTA) has been documented. The effect of the chelating agent EDTA on lead accumulation of root and aerial parts of growing seedlings of the plants *Amaranthus*, *Agropyron* and *Aeluropus* as been carried out and reported in this project. Although the application of EDTA strongly increased plant uptake of lead from contaminated soils, at higher metal concentrations the chelator was toxic to the plant. EDTA is nonbiodegradable in nature and very costly for large-scale phytoremediation processes, limiting its usefulness for long term soil cleansing. For that reason the effect natural chelators such as biohumus were studied. Biohumus contains a number of bioactive ingredients some of which chelate heavy metals. Though applied with approximately similar rates of application as conventional chemical fertilizers and capable of chelating heavy metals.

Materials and Methods

Plant waste processing looks more profitable if it can furnish products for the enhancement of food production. This approach has been used for the utilization of wheat straw. At the first stage the wheat straw was converted by oyster mushrooms into residual substrate and mushroom fruit bodies. The residual substrate was converted into biohumus by warms (Manukovsky et al., 1997).

Organisms. The cultures of oyster mushroom (Pleurotus florida Fovose) and californian worms (Eisenia foetida) were maintained at the laboratory.

Wastes. Stems, leaves and roots of potato (Solanum tuberosum L.); Wheat straw (Triticum aestum L.); Mixtures of the former two wastes (1:3, 1:1, 3:1 potato/straw (dry weight) were tested). Preparation of the substrates from the unused biomass of crops (fragment size 1-3 cm) was moistened to 70%, placed into cultivation vessels and sterilized for 2 h in steam at the pressure of 1.0 atm. The cultivation vessels were 3 liter glass jars. Each vessel contained 400 g dry weight of plant material mixture. After sterilization and cooling, the substrate in the jars was inoculated with oyster mushroom spawn. The jars were placed in an constant temperature incubator.

Sterile conditions were kept under spawn running. The glass jars with substrate and mature spawn were transported from the thermostat to the cropping chamber, a box with glass doors. The frame of the cropping chamber was made of stainless steel. The cropping chamber was supplied with a fluorescent lamp to initiate fruiting and with a sprayer to sustain the air humidity. Mushroom fruit bodies developed in the cropping chamber under non-sterile conditions. Two butches of mushrooms were harvested. The first was harvested between 50-60 days after spawn inoculation and the second between 70-80 days from inoculation. The overall time of oyster mushroom growing was about 80 days. Microclimate parameters under spawn running and fruiting were maintained according to the recommendations for oyster mushroom growing. Residual substrate was used to cultivate worms.

The residual substrate was transferred from glass jars to wood boxes 10 x 10 x 40 cm^3 and treated with adult worms at the rate of 5 worms per 100 g of wet substrate mass. The worms were cultivated for six months. The resulting mature biohumus was a mixture of residual substrate and worm casts. During this period moisture content of the substrate was maintained at 75-80%, at temperatures between 20-25 C.

The biohumus was then cleared of worms and filtered through a sieve (1.5 mm mesh). The filtrate obtained (biohumus preparation-1) was centrifuged at 10,000g for 10 min. Supernatant (biohumus preparation-2) after centrifugation was concentrated using a vacuum evaporator. The resulting material was again centrifuged and the supernatant separated into high and low molecular compounds by gel-filtration through coarse Sephadex G-

25. High molecular (biohumus preparation-3) and low molecular (biohumus preparation-4) fractions were tested by growing *Agropyron* and *Aeluropus* plants on them.

Pots of 15 x 15 x 15 cm^3 were used for plant growth. After two weeks of plants grpwtj, each pot received about 500 ml of four different biohumus preparations. Biohumus preparations were applied (i.e. fertilization with biohumus) as a solution to the soil surface using duplicate treatments with a control group that did not receive biohumus. Plants of *Agropyron* and *Aeluropus* were grown in groups of 30 plants per pot in a greenhouse with 14 h daylight, 25°-18°C day/night temperatures. The young seedlings were then fertilized daily with half-strength Hoagland's solution for 2 weeks. Uniform seedlings (15) were retained in each pot and the others eliminated. the pots were randomly divided into 5 groups (control and treatments), each group with 3 replicates. After 2 weeks, a solutions of chemical grade $Cu(NO_3)_2$ with different Cu^{2+} concentrations were supplemented to the growth media at the beginning of the treatment with biohumus preparations was added to the pots. The Cu^{2+} concentrations in the growth media were 0, 500 and 1000 μg g^{-1}(DW) for both the control and biohumus-treatments.

After adding the copper and biohumus supplement, the seedlings were grown for another 2 weeks. Then they were harvested and dried at 85° C. Dry mass of plants and copper content in different plant organs were determined.

Results and discussion

Hyperaccumulation of metals depends on several factors: most importantly, the genetic capability of the plant and metal bio-availability. Total heavy metal accumulated by the plant depends on the soluble soil copper levels. i.e. the accumulation of Cu from soils depends on maintenance of copper in a soluble form in the soil for better plant roots uptake and eventual translocation to the shoots. The solubility of copper in soil is limited due to complexation with organic matter, adsorption to clays and oxides. Increased solubility can be achieved by adding "detergents" to the soil.

For successful practical application of phytoremediation a number of approaches must be considered to solubilize soil heavy metals. Among the most important of these is the use of the biohumus. Biohumus produced from residual waste using Californian red worm contains huge amounts of bioactive micro- and macromolecules which may act as surfactants, and may replace root exudates. Thus, it can be hypothesized that the biohumus may play a role of detergents.

The use of copper containing soil (see Materials and Methods) in this study allows a more direct evaluation of the role of biohumus in the enhancement of Cu uptake as opposed to solubilizing soil copper with EDTA. Two-weeks old plants were transferred to the soil with biohumus and harvested two weeks after biohumus application. Roots and shoots were separated by cutting the stems approximately 1 cm above soil level and their weight and copper content were determined.

The roots of *Agropyron desertorium* were more sensitive to the high concentrations of copper (Table B-7). The presence of copper in concentrations up to 1000 _g/g in culture medium did not cause any visible change in the growth of *Aeluropus litoralis*. While an overall reduction of 40 % in biomass of plants was observed for *Agropyron desertorium* at copper concentrations near 1000 _g/g. Treatment with 500-1000 _g/g Cu for two weeks significantly diminished the biomass production in *Agropyron desertorium*.

Table B-7. Effects of biohumus preparation-1 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Agropyron desertorium* after next two weeks of culture in Cu-enriched medium.

Cu treatment μg/g	Biohumus treatment	Roots		Shoots	
		Biomass, g	Cu content, mg/g	Biomass g	Cu content, mg/g
0	-	17.8±3.5	0.130±0.062	79.5±8.8	0.027±0.006
	+	25.7±4.2	0.145±0.055	124.6±34.5	0.037±0.01
500	-	12.7±3.0	5.7±0.72	57.7±11.6	1.6±0.04
	+	24.8±5.3	12.8±2.8	119.0±20.3	3.7±0.8
1000	-	10.8±2.5	11.7±1.7	49.3±9.7	2.3±0.5
	+	25.0±6.2	23.9±5.3	122.3±34.1	8.4±2.3

Results are means ± SD

The response of plants to the applied biohumus preparation-1 was immediate, i.e. their growth rate and biomass production were increased. *Agropyron* grown in the soil with biohumus application produced more than twice the biomass of plants not receiving the biohumus (Table B-7). While the dependence of biomass production of *Aeluropus litoralis* on biohumus preparation was less impressive (Table B-8).

Table B-8. Effects of biohumus preparation-1 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Aeluropus litoralis* after next two weeks of culture in Cu-enriched medium.

Cu treatment µg g^{-1}	Biohumus treatment	Roots		Shoots	
		Biomass g	Cu content, mg g^{-1}	Biomass g	Cu content, mg g^{-1}
0	-	20.3±6.25	0.10±0.02	89.3±12.6	0.27±0.06
	+	26.4±8.4	0.12±0.02	125.7±21.0	0.28±0.08
500	-	27.2±8.3	0.24±0.05	102.6±20.3	22.3±6.3
	+	37.3±7.8	0.31±0.08	130.3±30.2	53.8±8.6
1000	-	22.5±6.2	0.28±0.1	100.7±21.4	38.4±8.2
	+	38.7±8.5	0.39±0.12	131.2±32.5	54.7±7.5

Results are means ± SD.

A positive correlation was found between application of biohumus preparations and Cu accumulation in both plant roots and shoots of *Agropyron* and *Aeluropus*. Most of the Cu accumulation was observed in the roots of *Agropyron desertorium* while most of the metal taken up by *Aeluropus litoralis* rppts were translocated to the shoots. The roots of *Agropyron* accumulated copper 3-5 times more than the shoots at 500 and 1000 µg g^{-1} concentration of the metal in the soil. At the same time the halophyte *Aeluropus* accumulated 92-140 times more copper in the shoots than the roots (Table B-7 and B-8).

Application of biohumus to soil containing 500-1000 µg g^{-1} Cu substantially increased Cu concentration in *Agropyron* roots and shoots, while it also stimulated plant growth. At concentration of copper 500 µg g^{-1} the heavy metal content in the roots of *Agropyron* rose from 5.7 mg/g for unfertilized specimens to 12.7 mg/g for plants fertilized with the biohumus, and in the shoots from 1.6 to 3.7 mg/g, respectively. When the copper supplied in the growth medium doubles (from 500 to 1000 µg g^{-1}), the metal accumulated in the root of *Agropyron* almost doubled (Table B-7).

At concentration of copper of 500 µg g^{-1}, the heavy metal content in the roots of *Aeluropus* rose slightly from 240 µg g^{-1} for unfertilized plants to 310 µg g^{-1} for those fertilized with biohumus, however the increase in the shoots was relatively more extensive from 22.3 to 53.8 mg/g, respectively. When the copper supplied in the growth medium was doubled from 500 to 1000 µg g^{-1}, the metal accumulation in the root of *Aeluropus* was negligible, however, in the shoots the difference in Cu content at 1000 µg g^{-1} of soil Cu concentration was double in the presence of the biohumus (Table B-8). Finally, the total content of copper accumulated un the roots and shoots of *Aeluropus litoralis* was 1.7 times higher than that of *Agropyron desertorium* showing the Cu-accumulation potential of the halophyte.

Table B-9. Effects of biohumus preparation-4 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Agropyron desertorium* after two weeks of culture in Cu-enriched medium.

Cu treatment µg g^{-1}	Biohumus treatment	Roots		Shoots	
		Biomass g	Cu content, mg/g	Biomass g	Cu content, mg g^{-1}
0	-	17.5±2.9	0.112±0.05	48.5±9.2	0.025±0.006
	+	22.7±4.8	0.125±0.05	119.3±245	0.030±0.005
500	-	11.9±3.0	5.6±0.82	56.9±10.6	1.6±0.03
	+	23.2±5.0	10.7±2.2	113.6±208	3.0±0.62
1000	-	10.2±2.5	10.9±1.9		2.2±0.63
	+	23.7±6.3	22.0±5.4	115.3±244	7.3±1.6

Results are means ± SD

Application of biohumus preparation-2 to the soils containing 500 and 1000 µg g^{-1} Cu showed results similar to those obtained using preparation-1 (data not shown). However, high molecular and low molecular compounds of the biohumus obtained by the separation of preparation-1 showed quite different effects on plant growth and copper accumulation. The effects of the presence of biohumus preparation-4 in growth medium on plant biomass and metal accumulation is described in Table B-9.

Like biohumus preparation-1, preparation-4 enhanced biomass production and copper accumulation in 4-week old *Agropyron* and *Aeluropus* plants grown in Cu-enriched medium. However, its effect was slightly lower than biohumus preparation-1, probably, due to its dilution after gel-filtration through the Sephadex. Thus, the biohumus preparations 1 and 4 not only increases the **biomass production** of the plants but also **Cu-accumulation** and protects against Cu-toxicity at its high concentrations (in the present experiments copper levels never reached a concentration which could produce necrosis). The increase of biomass production by biohumus preparation-4 was more pronounced in the roots of both plants than in their shoots. Root biomass increased almost 2 fold in the presence of preparation-4 while shoot biomass increased 1.4 fold.

Table B-10. Effects of biohumus preparation-4 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Aeluropus litoralis* after next two weeks of culture in Cu-enriched medium.

Cu treatment µg g^{-1}	Biohumus treatment	Roots		Shoots	
		Biomass g	Cu content, mg/g	Biomass g	Cu content, mg g^{-1}
0	-	20.4±6.13	0.10±0.02	90.7±12.3	0.028±0.007
	+	23.2±5.4	0.12±0.02	105.4±19.6	0.027±0.008
500	-	26.8±6.3	0.20±0.06	100.5±26.3	19.7±4.3
	+	37.0±9.5	0.28±0.07	125.3±20.7	40.2±7.6
1000	-	22.6±5.2	0.27±0.05	92.5±17.4	33.4±8.2
	+	37.8±8.5	0.37±0.12	127.5±22.6	48.3±7.5

Results are means ± SD.

Biohumus preparation-1, contains low molecular compounds considered potential nutrition for the plants and are also copper chelators. Metal-chelator complexes in biohumus preparations may easily be taken up by plant roots. To test this hypothesis, 2-week-old seedlings were grown for two weeks on Cu-enriched medium containing biohumus preparation-1. These plants (group-1, Table 11) were then collected from their growth medium and their root and shoot biomass and Cu-content were determined. The growth medium further used to grow another group of 2-week-old seedlings (group-2, Table 11). After two week growth, Group 2 seedlings were harvested, separated into roots and shoots and their biomass and Cu-content determined. Reuse of Cu-enriched growth medium containing biohumus preparation-1 only slightly increased root and shoot biomass and copper accumulation in *Agropyron* and *Aeluropus*. As a control, growth medium containing biohumus preparations was kept for two weeks without plant seedlings, and they were cultivated with third group of 2-week-old seedlings (group-3, Table 11). Plants of group-1 and group-3 have nearly the same biomass and Cu-content while the plants of group-2 exhibited considerable lower growth rate, biomass and metal content.

Biohumus produced from the stems, leaves and roots of potato and wheat straw contains low molecular compounds which can serve as plant nutrients and metal-chelators. Only low molecular fraction of biohumus is able to increase biomass production and copper accumulation in both *Agropyron* and *Aeluropus* plants (Tables B-9 and B-10). **Plants grown in biohumus-containing growth medium take up these compounds which disappeared from the medium after two weeks of plants growing in it (Table 11)**

Table B-11. Effects of the reuse of growth medium containing biohumus preparation-1 on biomass production and copper accumulation in the roots and shoots of *Agropyron* and *Aeluropus*.

Agrpyron desertorium				
Cu-treatment	Roots		Shoots	
µg/g	Biomass g	Cu content mg/g	Biomass g	Cu content mg/g
Group 1				
0	24.6±5.4	0.143±0.03	131 0±23.7	0.035±0 05
500	24.4±3.5	13.5±0.3	121.3±20.5	4 1±0.8
1000	24.7±6.3	21.7±6.6	119±25 0	8 6±1 3
Group 2				
0	18.7±3.3	0.065±0.0	80 4±15 6	0.023±0 004
500	13.4±2.6	3 4±0.4	60 2±9 7	1.2±0.2
1000	11.3±2.3	4.6±0.7	51.6±8.8	1 8±0 3
Group 3				
0	24.9±4.4	0.135±0.04	126.4±15.2	0.035±0 004
500	23.5±5.3	12.6±2.7	123.2±20 4	3 6±0 4
1000	23.7±5.4	20.3±4.7	125.0±34 2	7.5±0 6

Aeluropus litoralis				
Cu-treatment	Roots		Shoots	
µg/g	Biomass g	Cu content mg/g	Biomass g	Cu content mg/g
Group 1				
0	26.8±3.5	0.12±0.02	121 4±23 3	0.30±0 0
500	38.2±10.2	0.29±0.08	129.2±43.2	50 4±1 1
1000	40.5±8.6	0 35±0.07	130.6±23.7	55.3±18
Group 2				
0	19.7±3.5	0.08±0.01	90.6±20 4	0.25±0 0
500	25.3±4.8	0.20±0.05	98.8±9.7	18 0±4 3
1000	22.2±3.6	0 34±0.07	95 3±15 6	28.6±3.3
Group 3				
0	24.6±3.4	0.1±0.02	118.6±24.3	0 32±0 06
500	34.4±3.7	0 25±0 04	125.8±13 5	52 8±6.3
1000	36.0±7.5	0 34±0 08	127.0±24 4	56 0±14 0

Results are means ± SD.

Unlike biohumus preparation-4, preparation-3 was unable to increase biomass production and copper accumulation in roots and shoots of either *Agropyron* and *Aeluropus* plants (Table B-12 and B-13). Preparation-3 did not protect the plants against copper toxicity. Biomass production and copper accumulation were the same in the plants grown with or without biohumus preparation-3 (Table B-12 and B-13). Biohumus contains high molecular weight humic and fulvic acids which are recognized as strong metal-chelators. These acids probably bind copper preventing its uptake by plant roots.

Table B-12. Effects of biohumus preparation-3 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Agropyron desertorium* after next two weeks of culture in Cu-enriched medium.

Cu-treatment µg/g	Biohumus treatment	Roots		Shoots	
		Biomass g	Cu content, mg/g	Biomass, g	Cu content, mg/g
0	-	17.8±3.5	0.130±0.62	3.3±0.5	0.027±0.008
	+	18.2±3.5	0.145±0.55	80.3±24.5	0.027±0.01
500	-	12.7±3.0	4.50±0.72	55.6±10.6	1.6±0.14
	+	13.8±3.3	4.68±1.3	59.8±10.3	1.7±0.4
1000	-	6.8±2.5	10.7±1.2	22.5±5.7	
	+	6.4±2.2	11.4±2.3	23.3±4.7	3.4±0.8

Results are means ± SD.

We could assume that the humic and fulvic acids are natural chelating agents that reduce the avalability of metals to the plant roots. In order to test this hypothesis we carried out the following experiment. High molecular fraction (or biohumus-3) was incubated with100 mM

Table B-13. Effects of biohumus preparation-3 on the biomass production and copper accumulation in roots and shoots of 2-week-old *Aeluropus litoralis* after next two weeks of culture in Cu-enriched medium.

Cu-treatment µg/g	Biohumus treatment	Roots		Shoots	
		Biomassg	Cu content, mg/g	Biomass g	Cu content mg/g
0	-	20.3±6.5	0.10±0.02	89.3±12.6	0.27±0.06
	+	22.2±5.4	0.12±0.02	90.6±21.3	0.28±0.08
500	-	23.2±4.3	0.21±0.03	112.0±247	22.3±8.3
	+	23.0±3.8	0.22±0.06	115.3±242	22.8±6.6
1000	-	20.5±6.2	0.29±0.05	84.7±21.4	38.4±5.2
	+	20.5±5.5	0.31±0.07	86.2±32.5	40.7±7.5

Results are means ± SD.

$Cu(NO_3)_2$ was equilibrates with HMF at room temperature for 24 h. For separation of a high molecular fraction (HMF) from the excess of copper the mixture was gel-filtered through the Sephadex G-25 column equilibrated with 50 mM potassium phosphate buffer (pH 7.0) containing 50 mM NaCl. First HMF was used as a growth medium for one week-old seedlings of *Agropyron desertorium* and *Aeluropus litoralis*. The HMF separated from the excess of Cu(NO3)2 by gel-filtration contained 275 µg/ml copper. As a control HMF was incubated without Cu(NO3)2 for 24 h at room temperature and also gel-filtered through the same Sephadex column. HMF obtained from the gel was used as growth medium for control plants. Plant seedlings were grown on Petri dishes with filter paper for next one week.

Both Cu-pretreated and control HMFs were mixed with 10% ethanol. The ethanol treatment prevented the bacterial growth on HMF-medium and did not cause any visible change in the growth of the seedlings.

We observed substantially higher copper concentrations in plants grown in the water containing HMF-bound copper and EDTA, though soluble copper levels were only negligible in the complex of HMF-Cu

Table B-14. Effects of the presence of EDTA on biomass production and copper accumulation of plants grown in growth medium containing biohumus preparation-3.

Plants	EDTA treatment	Root		Shoot	
		Biomass, mg	Cu-content, µg/g	Biomass, mg	Cu-content, µg/g
Control-1: without HMF and Cu					
Agropyron desertortium	-	9.0 ± 3.2	3.0 ± 1.3	20.4 ±4.5	1.0
	+	5.6 ± 1.6	2.8 ± 1.2	12.8±2.3	1.2
Aeluropus litoralis	-	9.3 ± 2.7	2.5 ± 0.2	23.0±4.2	1.0
	+	5.0 ± 1.2	2.7 ± 1.4	13.5±3.3	1.3
Control-2: with HMF					
Agropyron desertortium	-	9.2 ±2.3	5.0 ±1.3	22.0 ±4.2	2.0 ±0.6
	+	7.5±1.3	5.1±1.6	17.8 ±3.5	18.3±6.3
Aeluropus litoralis	-	9.4±2.0	4.8±1.4	25.8±7.6	11.0±2.4
	+	8.7 ±2.2	4.4 ±0.9	20.2±4.3	10.2±0.3
With HMF and Cu					
Agropyron desertortium	-	9.2 ±2.5	5.2±1.5	22.4±4.0	2.3±0.5
	+	7.8±2.2	94.7±12.5	18.0±3.7	845.3±175.4
Aeluropus litoralis	-	9.4±3.2	4.7±0.8	22.5±5.3	18.3±3.6
	+	9.0±3.0	185.3±40.5	17.7±3.5	1256.6±178.3

Results are means ± SD.

In the absence the HMF biohumus and copper in the medium the biomass production of the plants is severely affected by EDTA, showing a dramatic decrease in biomass of both roots and shoots (Table 14, control-1). In the presence of EDTA the copper became available to plant roots and copper accumulation increased dramatically in both roots and shoots of *Agropyron* and *Aeluropus*. Results obtained show that affinity of EDTA to the metal is higher than that of biohumus.

When biohumus HMF is added to soil containing heavy metal, biohumus not only complexes the soluble Cu, but also due to the strong affinity of some compounds of biohumus for heavy metals, it solubilizes the metal tightly adsorbed to clay particles. Thus, the concentration of heavy metal in the soil available for plant uptake depends on the ability of biohumus to solubilize adsorbed Cu in the soil. A similar mechanism holds true for plants grown in Cu contaminated soils amended with biohumus. Assuming that after fertilization with biohumus, most of the soluble copper in the soil exists as its complex with a compounds of biohumus, increasing the concentration of soluble copper through applications of the biohumus to the soil should increase the Cu uptake by the plant.

An updated technology in phytoremediation of contaminated soils should include the use of natural complexing agents such as the biohumus in order to increase the solubility of toxic heavy metal ions. **More** importantly, biohumus not only facilitates heavy metal removal from the soil via plant uptake. The large scale use of *Aeluropus* for phytoremediation of heavy metals of soil and water, seems efficient and feasible. Plant with biohumus might be used for phytoremediation of low levels of heavy metals in soils. So, further studies to evaluate the rate of uptake after the biohumus application are required to substantiate this effect.

5.C Regulation of phytochelatin synthesis and accumulation in pea plants.

A more precise understanding of the biochemical mechanisms determining the suitability of a plant as a heavy metal hyperaccumulator was consider helpful for several purposes:

(a) To facilitate recognition of plant species with potential without having to go through long screening through pot and greenhouse experiments. The latter would be good to study a small number of species (3-5) but would require a lot of manpower to extend the study to tens of potential native hyperaccumulators.

(b) To recognize basic mechanisms of uptake, xylem loading, chalation or complexation and storage of heavy metals that bestow the capacity to take up and accumulate large amounts of HM while maintaining a good capacity for biomass production.

(c) Identify eventually special genes which regulate the above mention functions for their eventual isolation, cloning and transfer to other plant species to enhance their hyperaccumulating capacity.

Soils contaminated with heavy metals often show increased levels of more than one metal. An excess of heavy metals such as Cd, Pb, Zn, Ni, Cu and others in the soil become extremely toxic to plants making contaminated soils unsuitable for crop production, not only because of their lower fertility but also for the contamination of crop products with heavy metals. Some plant species possess a unique ability to adapt rapidly and evolve tolerance to toxic or even lethal levels of heavy metals. Plants can detoxify heavy metals ions by means of a family of heavy metal-binding polypeptides called (MBPs), metallothionins (MTs) and phytochelatins (PCs) which bind metal ions through closely spaced cysteine thiolate groups. This polypeptides and glutathione have been implicated in heavy-metal homeostasis in plants. Phytochelatins are produced in plants upon exposure to heavy metals. As soon as free metal ions are chelated, PC production is terminated. After metal binding, PCs seem to be degraded. PCs may serve as a biomarker for heavy metal toxicity.

Glutathion (GSH) plays several important roles in defence strategies of plants against environmental stress. GSH is the precursor of phytochelatines, which allow the plant to withstand supra-optimal concentrations of heavy metals. GSH often constitutes the major pool of non-protein reduced sulfur. Factors that may control the rate of GSH synthesis in plants are (1) the availability of cysteine, (2) feedback inhibition of glutamyl-cysteine synthetase (γ- ECS) by GSH and (3) the activity of glutathione synthetase (GS). A correlation has been observed between PC biosynthesis and the quantity of GSH in the tissue.

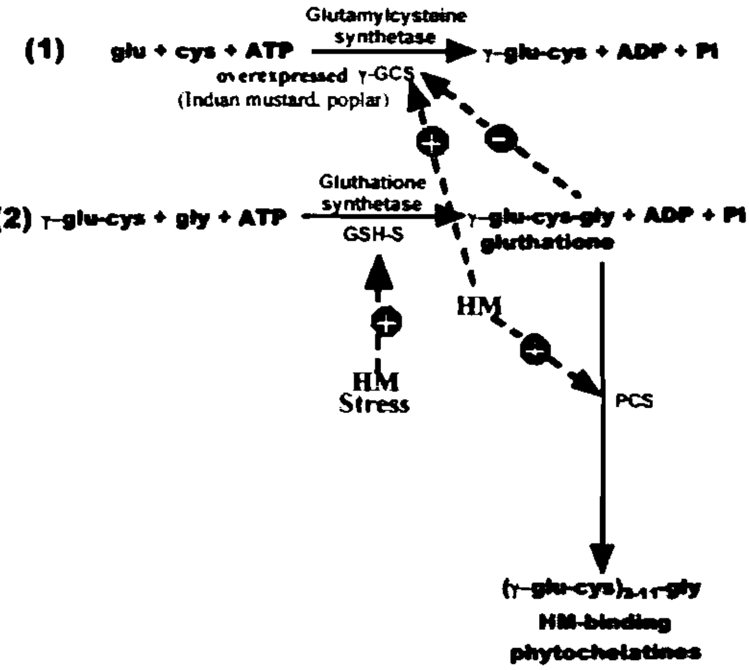

Figure C-1 Pathway of phytochelatine synthesis in plants

The chemical structure of PC bears close similarity to GSH. PC has the general structure (γ- Glu-Cys)n-Gly, with n=2-11. PCs are products of enzymatic biosynthesis. PC cannot be primary gene products that are synthesized on the ribosome because the γ-glutamyl linkage is not produced during translation and, in the case of iso-PC (β-Ala). Phytochelatin synthase (PS) adds the Glu-Cys from glutathione to another residue, resulting in the

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elongation of phytochelatin chain increasing the number of repeating units from n to n+1. Longer chain length seems to bind metals more tightly.

Plants vary in their response to stress factors and particularly to heavy metals. For inactivation of heavy metals plants synthesise phytochelatines. The increase of phytochelatin levels is usually preceded by enhanced levels of GSH in plant tissues.

As reported in previous annual reports of this projects several lines of biochemical studies were followed to study correlations between synthesis of GSH (gluthation) and the storage of HM in plants.
The highest level of GSH following heavy metal treatments was observed in pea plants after 7-9 days. GSH level in seedlings was highest after seven days of treatment with Cd. The level of GSH in seedlings was significantly higher than in roots of plants. Cd at concentration between 100 - 800 µM effectively stimulated synthesis of GSH in roots of pea seedlings. When Cu and Zn were used in nutrient medium no significant levels of GSH were observed in roots and seedlings of pea plants. Therefore Cu and Zn had less potent effect on GSH synthesis and accumulation in pea plants. Similar concentrations of Cd, Cu and Zn in nutrient solution were used to monitor the amount of ions of heavy metals accumulated in roots and seedlings of pea. Enhanced concentration of heavy metals was detected in roots of plants compare to seedlings.

Correlation was observed between GSH synthesis and the ability of roots to accumulate heavy metals in pea plants. Pea seedlings did not show significant accumulation of metals while GSH concentration was higher than in roots. Cd ions were most readily (50 ng/g DW roots) accumulated in plants compare to Zn (25 ng/g DW roots) and Cu (15 ng/g DW roots). Cd accumulation was observed after three days of exposure to heavy metal. For Cu and Zn this period was 14 days. The reason may have been a faster rate of uptake of Cd than Cu and Zn. This feature is presently being tested.
The effect of BSO ((L-Buthionine- sulfoximine), a potent inhibitor of Glutamyl cysteine synthetase) on GSH levels and Cd accumulation in several crops plants remarkably decreasing the level of GSH. Cd accumulation also decreased in roots in response on BSO treatment. Thus, GSH level in roots of plants in tightly linked with Cd accumulation.

Conclusions

1) GSH level in pea seedlings was highest after seven days of the addition of Cd to the nutrient solution. Cd treatment stimulates synthesis of GSH, the precursor of heavy metal binding proteins, in roots of plants at concentrations from 100 to 800 µM.

2) Cu and Zn were less effective on the enhancement of GSH synthesis and accumulation in pea plants. It will be interest to test is addition of Cd could enhance the removal of Cu and Zn.

3) There is a direct correlation between GSH synthesis and the ability of pea roots to accumulate heavy metals. Cd accumulated most readily (50 ng/g DW roots) in plants as compared to Zn (25 ng/g DW roots) and Cu (15 ng/g DW roots).

4) L-Buthionine-sulfoximine, a potent inhibitor of Glutamyl cysteine synthetase, decreased the concentration of GSH in the tissues as well as the accumulation of Cd in pea roots.

5D Stress induced responses of two wetland plant species potentially useful in phytoremediation

Introduction

Typha latifolia L. cattail	*Phragmites australis* (Cav.) Trin ex Steud. Common reed

Two wetland plant species: *Typha latifolia* and *Phragmites australis* were used in these experiments with the objective to study the effects of stress conditions on cysteine synthase (CS) and o-acetylserine thyol lyase (OASTL) activity and some related metabolic and defense pathways in plants (Hell, 1997). Thiol compounds are important in stress defense mechanisms. Glutathione (g-Glu-Cys-Gly) is a major source of thiol groups, which are strong electron-donors and therefore ideally suited for biological redox processes and in antioxidative responses during stress (Leustek and Saito, 1999). Glutathione is also the key element in phytochelatin synthesis, involved in detoxification and homeostasis of heavy metals in plants (Zenk, 1996). Over expression of OASTL conferred to plants increased tolerance of oxidative stress and this suggest that it may become possible to engineer stress resistant plants by manipulating cysteine synthesis (Saito, 2000). Also genetic engineering can produce useful plants for phytoremediation, in order to clean up polluted soil, water and air (Meagher, 2000). Reed and cattail are promising plants for these kinds of biotechnologies, as in experiments with cadmium both plants accumulated the toxic metal in their roots and defended themselves by increased synthesis of thiolic compounds.

Materials and methods

Experiments were carried out with reed (*Phragmites australis* Cav. Trin. Ex. Steud) and cattail plants (*Typha latifolia* L.) grown under controlled conditions. Plants were obtained by regeneration from rhizomes collected from local populations as well as plant micropropagated through tissue cultures.

Selected plants for experiments were grown in hydroponics with half strength Hoagland nutrient solution. In the case of controls, the nitrogen source was 2mM NO_3^-. Treated plants were grown for 2 weeks with: 2mM NH_4^+, 2.5- 100 µM Cd (as CdSO4) and 100 mM NaCl. Samples were taken several times during the treatments. OASTL (o-acetylserine thiol lyase) activity was determined spectrophotometrically, using the method described by Leon et al. (1988) and Barroso et al. (1998). In order to study the changes at transcriptional level of OASTL, the cDNA probe (kindly provided by Cecilia Gotor, Institute of Plant Biochemistry, Seville, Spain) was amplified in competent E.coli cells and the total RNA extracted from plants according to Brusslan and Tobin, (1992). The extracted RNA was transferred on nitrocellulose membranes for further hybridisation with the cDNA probe. Changes in the glutathione pool were recorded according to Law et al., (1983). Aldehyde oxidase (AO) activity was detected by native gel electrophoresis (Omarov et al., 1999).

OASTL activity was stimulated under different stress conditions mainly and firstly in roots in both experimental plants (fig.1, 2 and 3). Earlier results showed stimulatory effects in experiments carried out with detached leaves subjected to short Cd and salt treatments. Higher OASTL activities were recorded in Typha plants (fig 1). In different extend and different time periods NH_4^+, Cd and NaCl had the same stimulatory effect on OASTL activity.

Total GSH content remained largely unchanged in shoots but showed considerable change in roots. The increases and decreases of glutathione pool depend on time and on the kind of the stress too. The steady state of glutathione suggest that adaptive processes to stress conditions are functioning in the roots (May et al., 1998, Saito et al., 1994). The decrease of glutathione on Cd treated plants suggests that the incorporation of glutathione into phytochelatins, which bind the Cd and immobilizes the metal ions (Schafer et al., 1998). The increase showed the moment when, during stress conditions, glutathione is translocated from the shoots to the roots, to protect the plants against oxidative stress via the glutathione cycle. Previous measurements showed increase of total thiol pool in shoots shortly after treatments, while in the roots this increase appeared later.

Aldehyde oxidase activity increased in plants exposed to NH_4^+, Cd and NaCl . This stimulation increases in paralel with OASTL. It seems that both both sulfurylating enzymes, Cysteine synthase and Mo-hydoxylase sulfurylase, are regulated by the same factors. There may exist a relation between the two enzymes. a) dioxo-AO requires a sulfurylase in order to become active while OASTL activation is mediated by abscisic and thus depends on the activity of AO. This last idea has been discussed in recent papers (Barroso et al., 1999. Saito 2000).

General conclusions of the present stage of research:

- Key compounds in stress defense mechanisms such as cysteine synthase (OASTL), glutathione and total free thiol pool responded to increased levels of heavy metals in the nutrient solution of Typha and Phragmites plants subjected to stress conditions.
- OASTL activity varied with time and the following heavy metal concentrations in the nutrient solution: Cd (2.5-100 µM), NaCl (100 mM) and NH_4^+ (2 mM) as the only nitrogen source.
- AO activity correlated well with OASTL under stress conditions. They were regulated by the same factors (salinity, ammonium ions, heavy metals).

The efficiency of *Phragmites* and *Typha* in removal of Cd

Wetland plant species as *Phragmites* and *Typha* are useful tools for the remediation of eutrophic lakes, waste waters and contaminated soils. Plants in these experiments were subjected to long-term (56/140 days) Cd treatments (0-100 µM) in order to estimate their resistance to the metal and their ability to accumulate and transport it. Our results showed dependence on time and metal concentration. Interspecific differences were found between *Typha* and *Phragmites*. During severe stress *Typha* showed higher resistance. Higher efficiency in Cd removal and in translocation of the toxic metal to shoots are also observed in *Typha*.

Phytoremediation- "the green technology" – is a new field of life science, which uses plants for the removal of pollutants from the environment and their change into harmless forms (Gleba 1999, Salt et al., 1998). Plants possess many physiological, biochemical and genetic features contributing to their metal removing capacity: the ability of take up, transfer and store metal ions, the decomposition or biochemical transformation of xenobiotics, which make them ideal agents for bioremediation of soils and waters. Phytoremediation offers an efficient way to recover large extensions of soils and waters all over the world, polluted by human activities. This technology is a cost-effective and ecological sound alternative to remediation employing physical and chemical methods (Chaney et al., 1997, Meagher, 2000).

Apart from the organic pollutants, which can be completely decomposed and mineralized in plants, elemental pollutants are immutable by biological, physical processes and their remediation constitutes a special problem (Meagher, 2000). This category includes toxic heavy metal and radionuclides (As, Cd, Cs, Cr, Pb, Hg, U) and represents a major target for phytoremediation (Salt et al., 1998). Cadmium is an important environmental pollutant, present in areas with heavy road traffic, or near smelters and sewage sludge areas. Cd is present also as impurities in phosphate fertilizers. In some heavily polluted areas a content of 1-6 ppm Cd was reported (Haag-Kerwer et al., 1999).

Wetland species like common reed *Phragmites australis* (Cav.) Trin. ex Steud. and cattail *Typha latifolia* L. are supposed to be useful for the cleaning of eutrophic lakes, waste waters and contaminated soils (McNaughton et al., 1974, Taylor and Crowder, 1984). The present work is a comparative study of *Typha* and *Phragmites* plants concerning their ability to uptake and transfer Cd and their efficiency in the removal of toxic ions in hydroponic experimental models.

The accumulation of Cd depends on time and the severity of the applied treatment (fig. 1). Quantities of mg order were accumulated in roots in all experiments (fig 1) and in shoots during severe cadmium treatments (10-100 μM Cd), while μg amounts were recorded in shoots during mild stress (0.1-1 μM Cd) (fig. 1). *Typha* accumulates in time higher amounts of Cd in both roots and shoots. However after 14 and 56 days the determination of Cd content indicated small differences between *Typha* and *Phragmites* roots subjected to 100 μM Cd, the patterns of accumulation are different in the two species. The highest value of accumulation in *Typha* roots was 12±0.6 mg Cd/ g DW, achieved after 20 days of treatment and maintained with small changes till 75 days of experiment. Further increases in the accumulation of Cd in *Typha* plants continued through the 140 days of the experiment. The maximum accumulated rate in shoot was 1.01mg Cd/ g DW in *Typha*, achieved gradually by the day 75 of the experiment. Generally, in *Typha* the high rate of accumulation is achieved in short time after the start of treatments and maintained during the experiment, while in *Phragmites* the accumulated amount of Cd is significantly lower and changeable in time.

The percents of Cd transferred to the shoots (from the total Cd content of plants considered 100%) showed much higher ability of translocation in the case of *Typha* comparing to *Phragmites*. In *Typha* the percent of Cd transported to shoot was gradually increased in time; the highest value was 25.3% during the long-term treatment of plants with 10 μM Cd. During mild stress the translocation in *Typha* shoots showed much lower percents, without increasing with the time factor. In *Phragmites* subjected to severe stress the highest value was 3.6% of Cd transferred after 28 days of treatment with 100 μM Cd. Later the translocation is diminished. Long-term treatments with low Cd concentration (0.1 μM) of *Phragmites* plants led also to higher Cd translocation percent to shoot (4.3%).

Typha proved to be more resistant to Cd comparing to *Phragmites*, as plants subjected to severe stress (100 μM Cd) survived 140 days, while in the case of *Phragmites* only 56 days, in the conditions of our experiments. Leaves dried and the roots browned completely at the highest Cd concentration after the days indicated.

The efficiency of Cd removal was expressed as a ratio between the average Cd amount taken up by plants and the concentration applied (μg Cd g^{-1} DW /μM Cd). The analysis of data highlights that the efficiency of removal decreases as the applied concentration increases, in both species. The efficiency is in positive correlation with the time factor in all the treatments in the case of *Phragmites*. In *Typha*, during mild stress (01-1 μM Cd) efficiency showed small changes in time, while in the case of severe stress (10-100 μM Cd) the efficiency increased with time. Much higher efficiency was found in the case of *Typha* plants (maximum values ranging around 2000-3000μg Cd g^{-1} DW/μM Cd) comparing to *Phragmites* (800-1000 μg Cd g^{-1} DW/μM Cd).

Phragmites and *Typha* plants were subjected to long–term Cd treatments in order to estimate their resistance to the metal and also their ability to accumulate and translocate it. Our results represent total cadmium content of plants, resulted by both adsorption and absorption of the metal. For efficient phytoremediation both absorbed and adsorbed fractions are important (Meagher, 2000). In most plant species the main part of Cd is retained in roots and only a small percent is translocated in shoots (Trivedi and Erdei, 1992, Rauser and Meuwly, 1995, Lozano-Rodriguez et al., 1997, Ye et al., 1998). Our results support these data. Accumulation and translocation of Cd in *Phragmites*, according to our measurements are in agreement with data published by other research teams, showing high ability of retaining the metal in roots (Ye at al., 1997, Lakatos et al., 1999). *Typha* follows also this tendency, but accumulates higher Cd amounts and transports higher quantity to shoots comparing to *Phragmites*. The reason of Cd accumulation in roots is the binding of the heavy metal in phytochelatin complexes in cytosol and especially in vacuoles (Rauser, 2000, Cakmak et al. 2000). Literature data showed that 75-88% of Cd in most of the plants is retained in this form in roots.

Roots, immersed in nutrient solution, are in immediate contact with Cd, while shoots accumulate the metal taken up by the roots and transported through the xylem (Hart et al., 1998). This explains the differences in Cd content of roots and shoots and subsequently the differences in their responses to stress (Fediuc and Lips, submitted, Fediuc and Erdei, 2002, Fediuc et al., 2000). Interspecific differences between *Typha* and *Phragmites* have been showed previously, concerning their ability of take up and translocate Pb and Mb (Kufel, 1991). Different plant species as well as different genotypes of the same species manifested variability in Cd and other metals uptake and transport (Cakmak et al. 2000).

One basic required characteristic of plants used in phytoremediation is their tolerance to stress as growth inhibition in contaminated environments leads to low cleaning up efficiency (Zhu et al., 1999). Our previous results showed that both species possess protective mechanisms that enable them to resist stress conditions (Fediuc et al., 2000, Fediuc and Erdei, 2002). Naturally occurring Cd concentrations in polluted areas (0.1-10 µM) are tolerated by both *Typha* and *Phragmites* during long-term experiments. During very severe stress *Typha* showed a higher tolerance. Typha also showed higher Cd removal efficiency.

Concerning the usefulness of *Phragmites* and *Typha* in phytoremediation we can suggest that naturally occurring plants can be used in rhizofiltration (technique which use roots for the removal of contaminants from aqueous environment), while for phytoextraction (which requires the transport and accumulation of contaminants in harvestable organs, usually shoots, Chaney et al., 1997, Chardonens et al., 1999) *Typha* seems to be more feasible. Our results and suggestions are in agreement with those reported by Dinka (1986) based on Cd bioaccumulation in the same plants. Hyperaccumulators are able to accumulate higher Cd amounts in shoots. The efficiency of removal using *Typha* and *Phragmites* might be increased by genetic engineering for achieving higher accumulation and better translocation to shoot. These methods are more and more known and developed in our days (Gleba et al., 1999, Francova et al., 2001).

General conclusions of the present stage of research:

- Key compounds in stress defense mechanisms such as cysteine synthase (OASTL), glutathione and total free thiol pool responded to increased levels of heavy metals in the nutrient solution of Typha and Phragmites plants subjected to stress conditions.
- OASTL activity varied with time and the following heavy metal concentrations in the nutrient solution: Cd (2.5-100 µM), NaCl (100 mM) and NH_4^+ (2 mM) as the only nitrogen source.
- AO activity correlated well with OASTL under stress conditions. They were regulated by the same factors (salinity, ammonium ions, heavy metals).

6) Impact Relevance and Technology Transfer:

After two CAR projects the capability end the equipment facilities of the research group of Dr. Zerekbay Alikulovs has increased dramatically. This is directly reflected on the depth and quality of the research produced in his research unit and on the qualification of the doctoral students emerging from it. The AID/CDR project have been fundamental to help develop and effective, well motivated group of researchers that can tackle any problem related to agricultural productivity in arid lands and management of natural resources in Kazakhstan.

Left behind is a very good group of researchers, from which graduate and doctoral students are emerging continuously using a greatly improved laboratory equipped with many basic tools to carry out independent and fruitful research

7) Project Activities/outputs:

(a) Presentations at international science conferences

Moshe Sagi and Herman S. Lips
Salinity generated signals activate aldehyde oxidase and the synthesis of ABA
Dahlia Greidinger Conference on Mineral Nutrition. Haifa, Israel. March 1999

Rustem T. Omarov and S. Herman Lips.
The activities of enzymes related to stress adaptation in roots of barley as effected by inorganic nitrogen ions. Dahlia Greidinger Conference on Mineral Nutrition. Haifa, Israel. March 1999

Erdei, L. Szegletes, Zs., Csiszár, J., Barabás, N K., Lips, H., Sagi, M., Horváth V G., Oberschall, A., Dudits, D.
Physiological background of osmotic stress tolerance in transgenic tobacco plants overproducing aldose/aldehyde reductase. Israeli-Hungarian Conference on Plant Stress Biochemistry. Szeged Hungary. May 1999

Alikulov Z.A. and Boguspaev K.K. 2000. Perspectives and promises of phytoremediation in Kazakhstan. Biotechnology (Theory and Practice). N1-2, pp. 5-17.

Erdei, L., E. Fediuc and S. H. Lips.
Cadmium uptake and the induced protective mechanisms in two wetland species. Phragmites australis and Typha talifolia. European Union. Crete Workshop – Phytoremediation 2000. State of the Art in Europe. Crete. April 2000

Boguspaev K.K., Zheksembekova M.A., Alikulov Z.A. 2000.
Phytoremediation of heavy metal polluted soils by amaranth subspecies. Reports of Al-Farabi National University, Biological Sciences, N3 (11), pp.26-32.
Fediuc F., L. Erdei and S.H. Lips.
Wetland plant species responses to cadmium. FESPP (European Federation of Plant Physiology Societies). Budapest. August 2000
Zdunek E. and S. H. Lips
Site of synthesis, transport and accumulation of abscisic acid in *Pisum sativum* in response to suboptimal growth conditions. FESPP (European Federation of Plant Physiology Societies). Budapest. August 2000
Lips, S. H., E. Fediuc, R. Omarov and L. Erdei
A comparative study of sulfurylases involved in phytoremediation. InterCOST Workshop on Bioremediation Sorrento, Italy. November 2000
Alikulov Z.A. 2001.
Candidate plants in Kazakhstan for phytoremediation of heavy metals from contaminated soils. Prospects and Reality of Sustanable Development Ecosystems of the Aral Sea Region. Proceedings of International Conference supported by INTAS. 6-8 November, 2001. pp. 34-39
Lips S. H., E. Fediuc E., M. I. Soares, R. Omarov and L. Erdei
Studies on the regulation of enzymes involved on synthesis of ABA and phytochelatines.
European Union COST ACTION 837. Phytoremediation. Madrid, Spain. April 2001
Boguspaev K. K, Arystanova Sh. Alikulov. 2001.
The use of suspension cells culture of *Amaranthus paniculatus* in the selection for the tolerance to Cd International Symposium "Molecular mechanisms of genetic processes and biotechnology". p 198.
Moscow, November 18-21, 2001, Russia
Fediuc, E., L. Erdei andS. H. Lips.
Stress induced responses of two wetland plant species of potential use in phytoremediation. European Union COST ACTION 837. Phytoremediation. Madrid, Spain. April 2001.
Boguspaev K.K., Arystanova Sh., Sadykov Sh., Ivashenko A.T. 2001.
Accumulation of heavy metals in vegetative organs of amaranth (A.tricolor, A.paniculatus) during the plant development. Reports of al-Farabi National University, Biological Sciences N5. pp 37-41
Lips S. H., E. Fediuc E., M. I. Soares, R. Omarov and L. Erdei
ABA and Cd interaction on the regulation of phytochelatine synthesis enzymes
European Union COST ACTION 837. Constructed wetlands. Larnaca, Cyprus. May 2001
Lips S. H.
Characteristics of aldehyde oxidase regulation in different plant species.
Gordon Conference on "Molybdenum and Tugnsten enzymes". Oxford. UK. July 2001
Lips. S. H.
Inorganic N ions, phytohormones and plant adaptation to environmental changes
II International Symposium "Plants under Environmental Stress". Moscow, Russia. October 2001
Boguspaev K.K., Kasymbekov B., Alikulov Z.A.. 2002
An effective technology for soil bioremediation in Aral Sea Region. Reports of al-Farabi National University. Biological Sciences N1 (8) pp. 24-28.

(a) Publications based and related to work in the project

Alikulov Z.A. and Hakim A. 2003. Effects of EDTA on heavy metal accumulation by *Amaranthus*, *Agropyron* and *Aeluropus* plant species. Biotechnology (Theory and Practice) (in press)

Cramer, M. D., Z. F. Gao, and S. H. Lips. 1999. The influence of rhizosphere dissolved inorganic carbon on carbon and nitrogen metabolism in salinity-treated tomato plants. New Phytologist 142:441-450

Barabas, N. K., R. Omarov, L. Erdei and S. H. Lips. 2000. Distribution of the Mo-enzymes aldehyde oxidase xanthine dehydrogenase and nitrate reductase in maize (Zea Mays L.) nodal roots as affected by nitrogen and salinity. Plant Science 155, 49-58.

Baiseitova N. and Alikulov Z.A. 2002. Presown priming of plant seeds with molybdenum improves heavy metal tolerance of mono- and dicot plants. Reports of Euro-Asian National University. N4. 23-33

Boguspaev K.K. and Alikulov Z.A. 2003. Effects of the biohumus on heavy metal accumulation by *Agropyron* and *Aeluropus* plant species. Biotechnology (Theory and Practice) (in press)

Fediuc E. and S. H. Lips. 2002. O-acetylserine thiol lyase activity (OASTL) in *Phragmites* and *Typha* under as affected by salinity and heavy metals. (submitted)

Fediuc E. and S. H. Lips. 2002. Aldehyde oxidase in *Phragmites australis* under salinity and heavy metals (submitted)

Fediuc E. and S. H. Lips. 2002. The efficiency of *Phragmites* and *Typha* in removal of Cd from contaminated soil (Submitted)

Fediuc A., S. H. Lips and I. Soares. 2002. The germination of Onobrychis seeds collected in the Kyzil Kum desert areas contaminated with heavy metals (In preparation)

Lips, S. H., R. T. Omarov and M. Sagi. 1999 Inorganic N ions as signals for the activation of mechanisms of crop adaptation to environmental changes Nutrient Management under Salinity and Stress. Israel Institute of Technology. Ed.: A. E. Johnston. Pp. 31

Lips, S. H., M. Sagi and R. Omarov. 2000. Mo-enzymes at the crossroads of signal transmission from root to shoot. In: Nitrogen in a sustainable ecosystem from the cell to the plant. M. A. Martins-Loução and S. H. Lips, Eds. ISBN 90-5782-063-3 Buckhuys Publishers, Leiden, Netherlands. Pp 239-250

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Sarsenbaeva Zh. and Alikulov Z.A. 2002. Effects of heavy metals on the activities of root molybdoenzymes of mono- and dicot plants. Poisk. N3: 45-52.

Zdunek, E., O. Falik, K. Fülöp, M. Gersani and S. H. Lips. 2000. Interchange of shoot and root signals during competition and limiting nitrogen. In: Nitrogen in a sustainable ecosystem from the cell to the plant. M. A. Martins-Loução and S. H. Lips, Eds. ISBN 90-5782-063-3 Buckhuys Publishers. Leiden, Netherlands. Pp. 205-210

8) Project Productivity:

The project has achieved numerous goals, if not as originally planned at least through alternative ways that looked more fruitful as the research developed. The main product is that the knowledge to start large scale phytoremediation of contaminated fields is basically at hand, although still in need of further optimization especially on the adaptation of the knowledge acquired to effective operation conditions in the field

9) Future Work:

The knowledge generated by this project sets a firm basis to carry out an in-depth pilot field experiment on soils contaminated with heavy metals to determine the interaction between native hyperaccumulator species, irrigation and fertilization to optimize HM removal from soils. Funding sources are now being sought within the international agencies operating in Central Asia and with the Government of Kazakhstan. This pilot project should last around 1-2 years.

After the pilot a large scale field run should be carried out, over an area of several Ha. Plants species that accumulate the HM taken up from the soil in the leaves and that show a good rate of regrowth after harvest, will be used preferentially although use of other hyperaccumulators is not discarded

At the same time the Alikulov group in Astana will continue its excellent research on native hyperaccumulators physiological and biochemical characteristics and identifying methods to facilitate their uptake of HM from contaminated soils. In all these endeavors they will find full collaboration from Israeli scientists and agronomists

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