ESRF	Experiment title: **Studies of thiol mediated cadmium and mercury uptake in plants for enhancing phytoextraction efficiency**	**Experiment number**: LS-2209
Beamline: BM23	**Date of experiment**: from: 19.6.2013 to: 24.6.2013	**Date of report**:
Shifts: 15	**Local contact(s)**: Gleb Parakhonskiy	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Iztok Arčon[a, c*], Katarina Vogel-Mikuš[b*], Alojz Kodre[c*], Jana Padežnik-Gomilšek[d*], David Jezeršek[e*], Mateja Potisek[b*], Eva Kovačec[b*], Wojciech Przybylowicz[f*], Isabelle Lefevre[g*], Charlotte Poschenrieder[h]

[a] University of Nova Gorica, Vipavska 13, POB 301, SI-5001, Nova Gorica, Slovenia
[b] Biotechnical faculty, Dept. of biology, Večna pot 111, SI-1000 Ljubljana, Slovenia
[c] Jozef Stefan Institute, Jamova 39, POB 000, SI-1001 Ljubljana, Slovenia
[d] Faculty of Mechanical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor, Slovenia
[e] Sincrotrone Trieste S.C.p.A Strada Statale 14 - km 163,5 AREA Science Park, Basovizza, Italy
[f] Materials Research Group, iThemba LABS, PO Box 722, Somerset West 7129, South Africa
[g] Groupe GRPV, 5 (Bte 13) Place Croix du Sud Louvain la Neuve, Belgium
[h] Universidad Autónoma de Barcelona, Facultad de Biociencias, Lab. Fisiología Vegetal. E-08193 Bellaterra, Spain

Report:

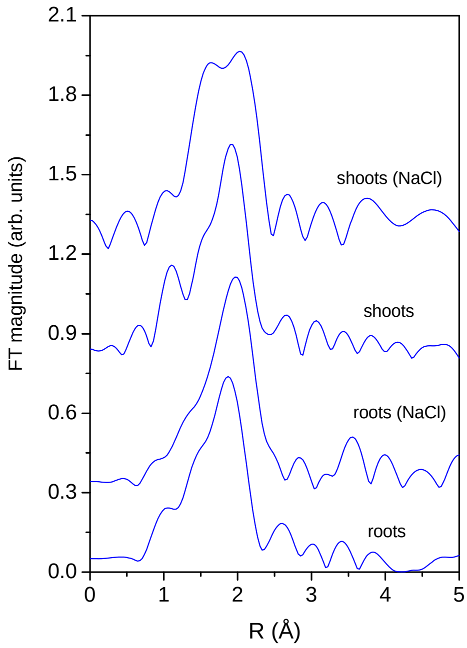
Hg and Cd are two of the most toxic and hazardous metals found in environment, known to accumulate in food webs with a biomagnification pattern at successive trophic levels [1,2], posing threads to human and animal health. Therefore their concentrations in crop plants need to be kept at minimum.

Phytoremediation is a direct use of green plants and their associated microorganisms to stabilize or reduce contamination in soils, sludges, sediments and surface or ground water [3]. Plants can be used either for removal of hazardous metals from soils (phytoextraction) or for the reduction of the mobility of heavy metals in soil (phytostabilizytion). The use of both techniques is, however, hindered by the lack of knowledge of the basic metal uptake and transport mechanisms, as well as by the limited number of suitable plant species. Metal hyperaccumulating plants are able to concentrate high amounts of metals in their shoots, but with few exceptions - such as for example Ni hyperaccumulators from the *Berkheya* genus [4], they are mostly low biomass crops. In parallel to the use of hyperaccumulating plants chelate-assisted phytoextraction, which uses different organic molecules as metal ligands in soil, thereby enhancing metal uptake into high biomass crop plants, proved to be an efficient alternative, especially for Hg removal [5]. On the other hand phytostabilization aims to remediate highly polluted areas, where phytoextraction is not feasible. The mobility of metal contaminants can be reduced by accumulation in plant roots, absorption onto the roots, or precipitation within the root zone [3].

Within obtained beamtime we have analysed Cd, Hg, Se and Ni ligand environment is selected plant species. For determination of Cd, Hg and Se ligand environment we have used freeze-dried plant material in order to ensure that metal concentrations were high enough to obtain good signal to noise ratio [6]. After harvesting the plants were rapidly frozen in liquid nitrogen and freeze-dried. Dried material was grind in a mortar with liquid nitrogen and pressed into self standing pellets which were mounted on the holder. XANES and EXAFS spectra were measured in cryo conditions, using He cryostat.

For Ni fresh plant material was used. Roots and leaves were detached from the plants and sticked on kapton tape, rapidly frozen in liquid nitrogen and mounted on the cryostat holder. Ni K-edge EXAFS spectra were recorded in cryo conditions, using He cryostat.

Metal accumulation in plants is closely connected to physiological and biochemical adaptations of the root metal uptake, metal transport from the roots to the shoots, and metal sequestration and detoxification in leaves [7]. Assuming that plants possess mechanisms to transport thiol-metal complexes across the plasma membrane [8], external addition of sulphur/thiol compounds might enhance Cd and Hg entrance into the symplast and transport of Hg- and Cd-thiol complexes to the shoots, facilitating phytoextraction procedure. The aim of this study was therefore to investigate Cd and Hg ligand environment in plants grown in hydroponic solution supplemented by 10 or 100 µM of Hg or 100 and 300 µM of Cd, 0.52, 0.62 and 0.82 mM of anorganic sulphur (sulphate) or 0, 0.25 and 0.5 mM of glutathione or cysteine, for four weeks. Plants treated with glutathione died after two weeks of treatment therefore they were eliminated from further analyses.



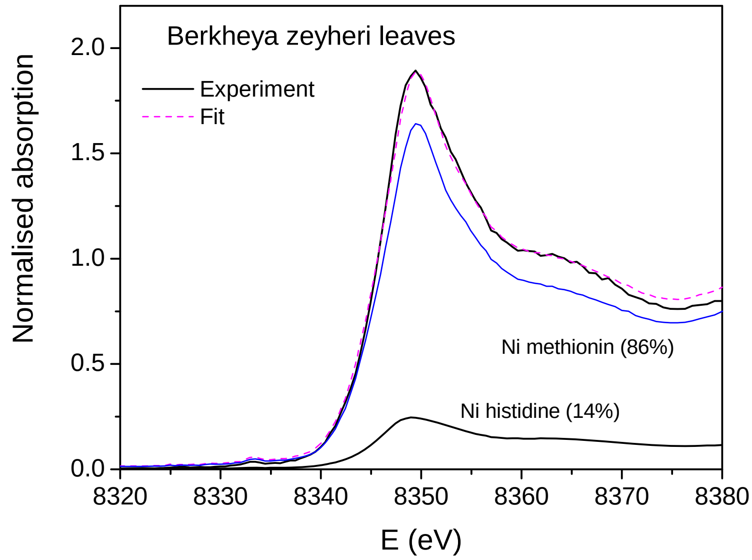


Figure 1: **Left:** FT magnitude of k^2 weighted Cd K-edge EXAFS measured on plant roots and shoots treated with Cd or simultaneously with Cd and NaCl. **Right:** Linear combination fit of Ni K-edge XANES measured on hyperaccumulator *Berkheya zeyheri* leaves with XANES spectra of reference Ni complexes Ni-histidine and Ni-methionine.

Cd ligand environment was also examined in halophyte plant *Sesuvium portulacastrum* that accumulates huge amounts of salt and water in the vacuoles of its succulent stems and leaves. The extreme salt tolerance of euhalophytes resides in the accumulation of NaCl along with other inorganic ions and water in the vacuoles. For compensating the low osmotic potential of the vacuole, compatible organic solutes are accumulated in the cytoplasm. It has previously been shown that *S. portulacastrum*, when grown in presence of 50 µM Cd in combination with 100 or 400 mM NaCl, can accumulate and tolerate more than 200 and 500 mg Cd kg^{-1} dry weight in its shoots and roots, respectively, without exhibiting Cd toxicity symptoms [9]. *S. portulacastrum* plants were grown for 4 weeks in basic nutrient solution (NS) supplemented by 50 µM of Cd (in the form of $CdCl_2$) in combination with 1 mM (control) and 100 mM NaCl. Cd K-edge EXAFS spectra were recorded in roots, stems and leaves of Cd and Cd+NaCl treated plants (Fig 1. Left).

Nickel ligand environment was examined in a Ni hyperaccumulator *Berkheya zeyheri* that is endemic for South Africa [4]. Plants were collected together with rhizosphere soil in natural environment and transferred to the pots. The plants were kept in green-house until the experiment. Ni K-edge XANES (Fig. 1. Right) and EXAFS spectra were recorded in the system soil-roots-stems-leaves and sap feeding insects.

Foliar treatment of plants with selenium proved to enhance Hg uptake into the plants roots, while translocation of Hg to the shoots was not affected. Selenium metabolism is intimately related to that of sulphur, since Se behaves as sulphur analogue in plant tissues. Corn was chosen as a model plant species and treated with 50 ppm of Hg (as $HgCl_2$ spiked with the substrate for growing plants). Simultaneously the leaves of a half of Se treated plants and the controls (without Hg) were sprayed with K_2SeO_4. Hg and Se ligand environment was examined by measuring Se K and Hg L_3-edge XANES and EXAFS spectra (Fig. 2), while sulphur metabolism will be studied through S K-edge XANES at XAFS beamline of Synchrotron Elettra.

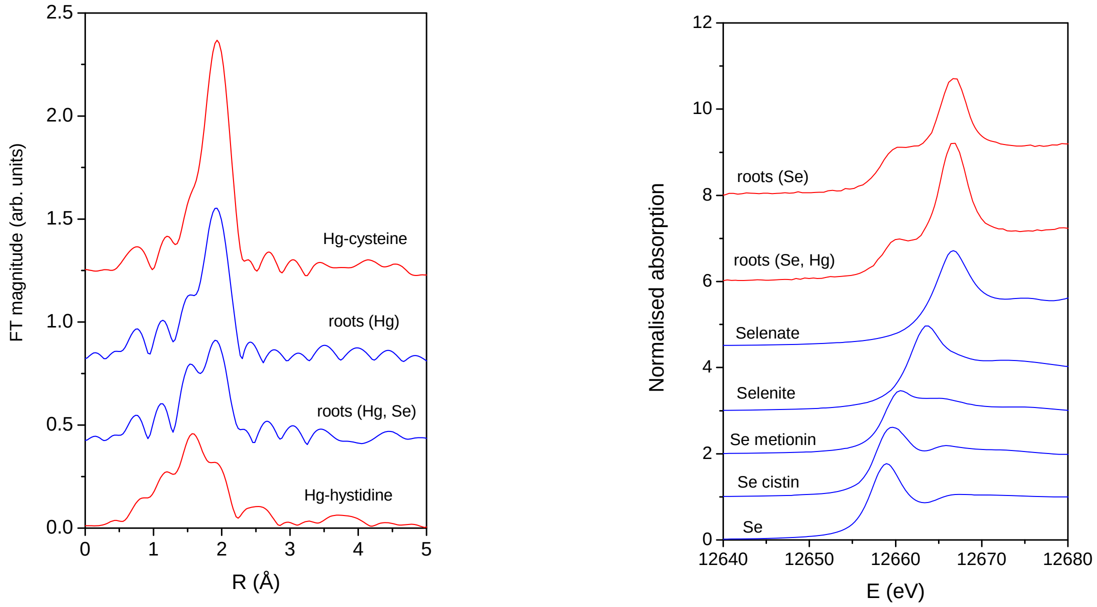


Figure 2: **Left:** FT magnitude of k^2 weighted Hg L_3-edge EXAFS measured on plant roots treated with Hg or simultaneously with Hg and Se and on reference complexes Hg-cysteine and Hg-histidine. **Right:** Se K-edge XANES measured on same plant roots and reference Se compounds.

The quantitative XANES and EXAFS analysis of Cd, Hg, Se and Ni spectra is in progress to obtain ligand environment of these elements in plant tissues.

References

[1] D. C. Adriano, *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals* (Springer-Verlag, New York, N.Y, 2001).

[2] J. Godt, F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich, and D. A. Groneberg, Journal of Occupational Medicine and Toxicology (London, England) **1**, 22 (2006).

[3] H. Ali, E. Khan, and M. A. Sajad, Chemosphere **91**, 869 (2013).

[4] K. Turnau and J. Mesjasz-Przybylowicz, Mycorrhiza **13**, 185 (2003).

[5] C. Lomonte, A. Doronila, D. Gregory, A. J. M. Baker, and S. D. Kolev, The Science of the Total Environment **409**, 2685 (2011).

[6] Š. Koren, I. Arčon, P. Kump, M. Nečemer, and K. Vogel-Mikuš, Plant and Soil (2013).

[7] C. S. Seth, The Botanical Review **78**, 32 (2011).

[8] S. K. Yadav, South African Journal of Botany **76**, 167 (2010).

[9] T. Ghnaya, I. Slama, D. Messedi, C. Grignon, M. H. Ghorbel, and C. Abdelly, Chemosphere **67**, 72 (2007).