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Report:

Cd pollution of biosphere results mainly from Cd-containing fertilizers, smelting and sewage sludge disposal. Cd is extremely toxic even at low concentrations, and it is easily taken up by plants and translocated to the food chains (1). The use of metal hyperaccumulating plants has appeared as a promising way for metal removal from soil (2), but more knowledge on the metal accumulation and detoxification is needed to understand soil-plant interactions for the use of phytoextraction in practice (2,3).

In this study we used X-ray absorption spectrometry (XAS) to obtain direct information on local structure around Cd cations bound in different tissues of Cd (hyper)accumulating species *T. praecox* and *Zygophyllum fabago* and in non-accumulating *T. arvense*. In previous research we found that *T. praecox* massively accumulates Cd in shoots (up to 5960 µg/g dry weight) and also in the seeds (up to 1351 µg/g dry weight), without drastically affecting plant performance and seed viability (4, 5). Micro-PIXE (proton-induced x-ray emission) localization studies of shoots and seeds showed that Cd is more or less evenly distributed through the leaf tissues with highest concentrations found in mesophyll symplast. On the other hand in seeds Cd is

mainly accumulated in the epidermis of cotyledons, away from future photosynthetically active embryonic tissues (5, 6).

Zygophyllum fabago was proved to be able to proliferate into coarse mineral substrates contaminated with heavy metals. It accumulates and tolerates up to 160 μg Cd /g dry weight (7) and like *T. praecox* it often accumulates also other toxic metals like Zn and Pb, which may be present in the polluted soil.

We measured Cd, Zn K-edge and Pb L_3–edge EXAFS and XANES spectra of different plant organs (roots, stems, leaves, seeds) and tissues, (mezophil, epidermis, veins) of hyperaccumulating species *T. praecox, Zygophyllum fabago* and non-hyperaccumulating *T. arvense* to determine chemical coordination of these metal cations (species and number of neighbors in the nearest coordination shells around metal cations, their distances and Debye-Waller factors). This information is expected to provide better understanding of types of complexes that are created when toxic elements are absorbed in plants (3).

The plants were either collected at Pb, Zn and Cd polluted site in Slovenia (*T. praecox*), grown in nutrient solution with added Cd and sulphur (+S) or with added Cd and depleted in sulphur (-S) (*T. praecox* and *T. arvense*) or with added Cd and/or Zn (*Z. fabago*). After harvest, plant organs were frozen in liquid nitrogen, freeze-dried, homogenized and packed in specially designed 9 cm or 5 cm long plastic holders for Cd K-edge EXAFS or pressed in homogeneous pellets for Zn K-edge or Pb L3-edge EXAFS experiment. In addition, EXAFS and XANES spectra of some reference Cd, Zn and Pb compounds with known metal valence state and structure, prepared in the form of homogenous self standing pellets, were measured in transmission mode for comparison. In all cases the optimal total absorption thickness of about 2 above the investigated absorption edge was obtained, with Cd, Zn or Pb edge jumps in the range between 0.1 to 1.5, depending on the concentrations of the investigated metal (ranging from about 100 to 10 000 μg/g dry weight), determined in advance by XRF.

The BM 29 station of ESRF was equipped with a Si 311 two-crystal monochromator with 2 eV resolution in the region of Cd K edge (26 711 eV) and about 1 eV resolution in the region of Zn K edge (9 659 eV) and Pb L3-edge (13 035 eV). Higher harmonics were eliminated by a flat Pt coated mirror placed after the monochromator for Cd K-edge experiment, and by a flat Si coated mirror for Zn and Pb experiments. The absorption spectra were measured in transmission detection mode. The intensity of the monochromatized beam was monitored with three ionization detectors, filled with krypton to the pressure of 120 mbar, 420 mbar, and 450 mbar, respectively, for the Cd K-edge measurements. In case of Zn K-edge measurements, the cells were filled with argon to the pressure of 60 mbar, 310 mbar, and 310 mbar, respectively, and for the Pb L3-edge measurents.to the pressure of 110 mbar, 710 mbar, and 900 mbar, respectively. In all cases He was added to the total pressure of 2 bar.

Samples were placed between the first pair of detectors, and the reference metal foils between the posterior pair, to check the stability of the energy scale. The absorption spectra were measured within the interval [-250 eV ..1000 eV] relative to the investigated absorption edge. In the XANES region equidistant energy steps of 0.5 eV were used, while for the EXAFS region equidistant k-steps ($\Delta k \approx 0.03$ Å^{-1}) were adopted, with an integration time of 1s/step. The absorption spectra of three identical runs were routinely superposed to improve the signal to noise ratio and to check the stability and reproducibility of the detection system.

Preliminary Cd EXAFS analysis show that in all plants mixed S and O ligands are present in the first coordination sphere. In some cases signal from backscattering of the photoelectron from carbon and phosphorous atoms in the second coordination sphere is also detected. FT EXAFS spectra (example on Figure 1 left) clearly reveal differences in Cd bonding in different plant tissues. The prevailing structural difference between different plant tisues obtained in the quantitative EXAFS analysis (example on Fig. 1. Right) is found to be in the number of S and O atoms in the first coordination sphere. The ratio of the two ligands in different tissues reveals the strength of Cd bonding and indicates the Cd complex responsible for Cd immobilisation. The S ligands most probably steam from thiol groups (e.g. glutathion) bonded to Cd cation, while O ligands may be ascribed to Cd cations bonded to hydroxyl and carboxyl groups of the cell wall components and organic acids found in vacuoles. Larger average number of S ligands is found in stems which indicates that Cd is probably transported from roots to shoots as Cd-thiol (Cd-glutathion) complex.

A detailed analysis of EXAFS data and the preparation of a publication is in progress. The obtained data is crucial for further tissue specific research on Cd, Zn and Pb transporters and/or antioxidant-based defense

and to understand molecular-level detoxification mechanisms that are efficiently contributing to heavy metal tolerance in (hyper)accumulating species.
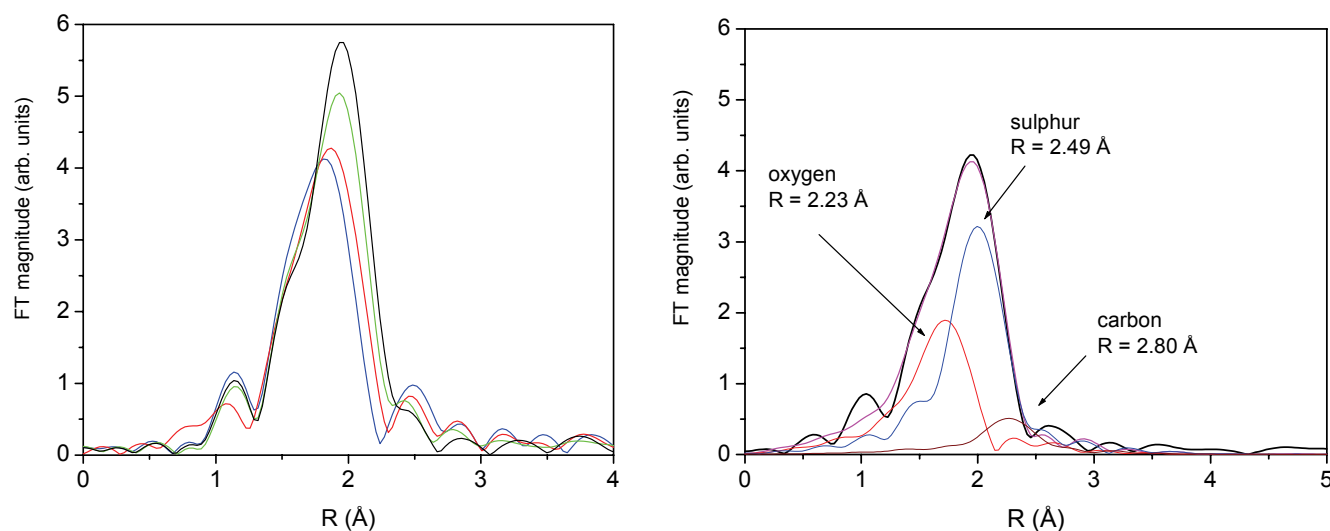

Figure 1: **Left:** Fourier transform of k^3-weighted Cd EXAFS spectra of of *T. praecox* leaves (+ S) - red**,** (*T. praecox* stems (+S) - green, *T. praecox* stems (-S) - black and *T. arvense* roots (+S) - blue). **Right:** Fourier transform of k^3-weighted Cd EXAFS spectra of *T. Praecox* shoots, calculated in the k range of 2.5–14 $Å^{-1}$. Experiment – (black line); EXAFS model – (magenta line). Contributions of O, S and C neighbors are plotted with red, blue and brown lines respectively.

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