ESRF	**Experiment title:** Depicting the role of externaly supplied organic sulphur compounds in Cd uptake, translocation, accumulation and detoxyfication in Cd hyperaccumulating and non-accumulating plants	**Experiment number**: EC-968
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Report:

Thlaspi praecox is able to hyperaccumulate more than 5000 μg g^{-1} dry weight (DW) of Cd in leaves under field conditions [1]. Metal hyperaccumulation 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. Over-expression of different metal transporters in roots together with low degree of vacuolar compartmentalization in root cortex cells predispose metal xylem loading in hyperaccumulating plants [3,4]. The majority of Cd and Zn in xylem sap was however proved to exist in free ion form or bound to small organic molecules like citrate, nitrate and hystidine [5] which, when abundant, facilitate metal transport to the shoots. The role of sulphur rich organic and inorganic compounds in root to shoot transfer of Cd in hyperaccumulating plants remains largely unresolved. Our preliminary study (performed at ID 21 ESRF, project EC-719) of the Cd distribution patterns in leaves of hydroponically grown plants of Cd hyperaccumulator *T. praecox* supplied with 100 or 300 μM of $CdSO_4$ showed that with increased tissue concentrations of Cd the proportion of sulphur ligands found in leaf mesophyll increases, probably in order to protect sensitive photosynthetical processes. Thiol-mediated Cd and Pb transport was recently described in *Brassica napus* and *Zea mays.* Authors suspected that thiol mediated transport is facilitated by one or more peptide transporters present in the plasma membrane that recognize metal-glutathione or metal-cystein complexes [2], however according to our knowledge the exact mechanisms of thiol mediated Cd transport are still not exactly clear, especially in Cd hyperaccumulating plants. Identification of the mechanisms and the roles of sulphur inorganic and organic compounds in Cd root trafficking and translocation would significantly contribute to the knowledge of the basic Cd uptake and transport mechanisms and help to develop more efficient phytoextraction technologies.

The aim of this experiment was to depict how sulphur metabolism in roots respond to Cd supply in plants fed with inorganic (SO_4) and organic (cystein) forms of sulphur or low sulphur supply, and whether Cd ligand environment and distribution change in plants fed with different sulphur compounds. Combination of 2D

elemental imaging, Cd-L_{III} micro-XANES, S-K micro-XANES and sulphur overedge mapping was employed.

T. praecox plants were grown in hydropnics in nutrient solution supplemented by 0, 100 µM and 300 µM of Cd (in the form of $CdCl_2$) and 0.25 (lowSO_4), 0.5 mM of SO_4 (SO_4) or 0.25 mM of SO_4 and 0.25 mM of cysteine (+Cys) for four weeks. At the end of experiment bulk element (P, S, Cl, Cd) concentrations were determined by energy dispersive X-ray spectrometry (results not shown).

The cuttings of plant roots were prepared using cryo-fixation and cryo-sectioning at -35 °C. The sections were mounted on holders and kept at -196 °C till the measurement, which was performed in cryo conditions at -196 °C. The 2D elemental mappings was performed at the ID 21 beamline using their SXM set-up equipped with cryostat. The X-ray beam delivered by the undulator was monochromatized by means of Si double crystal monochromator and focused to a submicron probe (0.7 x 0.3 μm^2) using zoneplate focusing. The fluorescence emission of the sample was collected by the SDD detector. The excitation energy for the scan was first set to 3.55 keV (i.e above the Cd- L_{III} edge) to record maps of Cd, S and P and at the same time below the potassium K edge in order to avoid the interference betwen Cd-L_{III} and strong potassium Kα signal. In selected regions of root sections of the same treatments S-K XANES and sulphur speciation by means of chemical contrast mapping at the sulphate (2.482 keV) and at the thiol (2.473 keV) peak energy was performed, since the ratio between the two gave us the relative amounts of each sulphur chemical state in plant tissues. Finally the Cd L_{III} – edge XANES spectra were recorded in different plant tissues and cells, depending on local Cd concentrations, to determine Cd chemical environment. CdL_{III}–edge XANES and S-K XANES spectra of standard reference materials were also measured.

Sulphur distribution in Cd treated plant roots depended on S supply. In Cys supplied plants S was localized mainly in central cylinder, while in SO_4 and low SO_4 supplied plants, S was concentrated more in rhizodermis (especially in low SO_4 plants) and outer cortex and colocalized with Cd (**Fig. 1, c, d, e**).

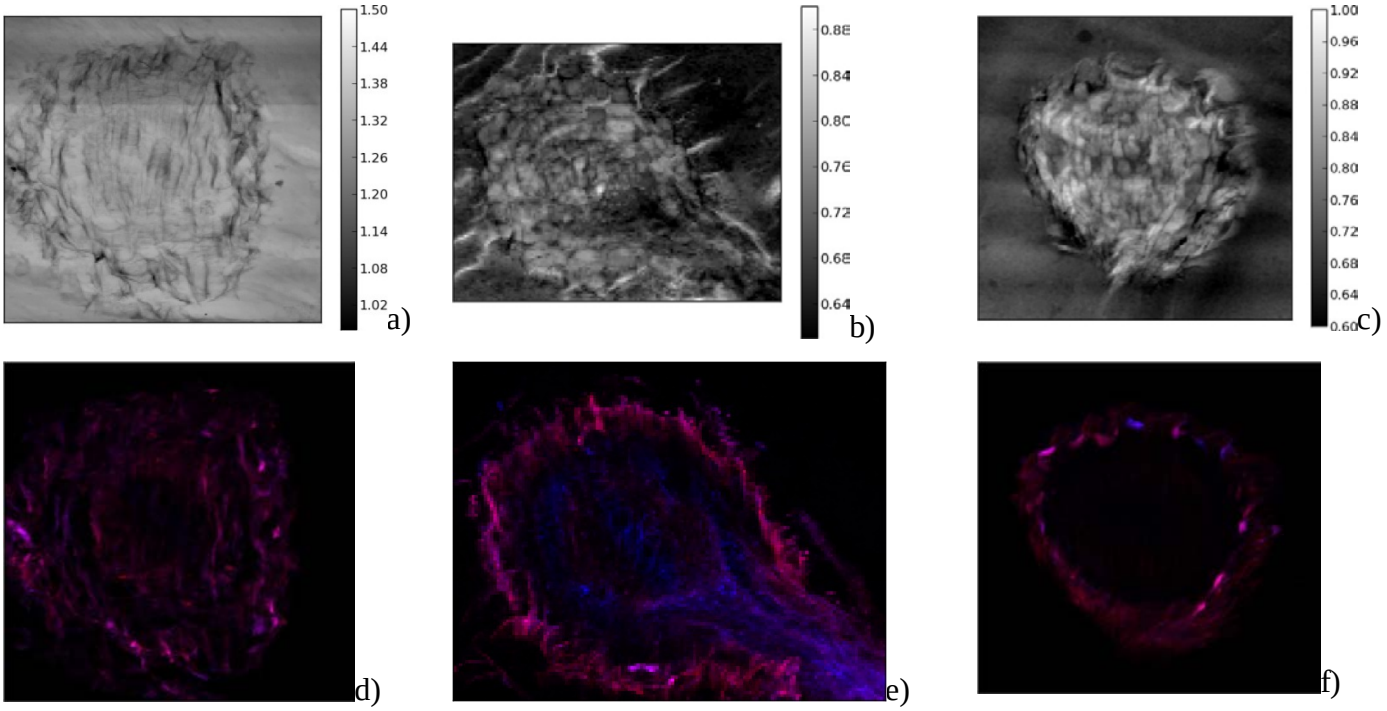

Figure 1: First panel: absorption images of cryofixed *T.praecox* root samples a) 500µM SO_4 (+SO_4), b) 250 µM Cys + 250 µM SO_4 (+Cys), c) 250 µM SO_4 (low S). Second panel: Distribution of Cd (red channel) and S (blue channel) in 100 µM Cd treated *T. praecox* root cross-sections d) 500µM SO_4 (+SO_4), e) 250 µM Cys + 250 µM SO_4 (+Cys), f) 250 µM SO_4 (low S). Purple colour indicates colocalization of S and Cd.

The preliminary S-K micro XANES results show that roots respond to Cd treatment with increased synthesis of -SH groups, especially when fed with cysteine (+Cys) and supplied with low S concentrations, since the first peak from the left which corresponds to -SH groups is significantly higher in Cd treated then in the non-treated plants (**Fig. 2b**). Plants usually take up sulphur in the form of -SO_4, which is also a storage form of

sulphur in plant tissues and then enzymatically reduce it to -SH which is then incorporated in amino acids as cystein and methionine. In sulphur starved plants the ratio between storage (SO_4) form and -SH forms was significantly shifted to -SH form production, which was especially apparent in Cd treated plants, where almost no SO_4 form was present in the root tissues (**Fig. 2a, 2c**).

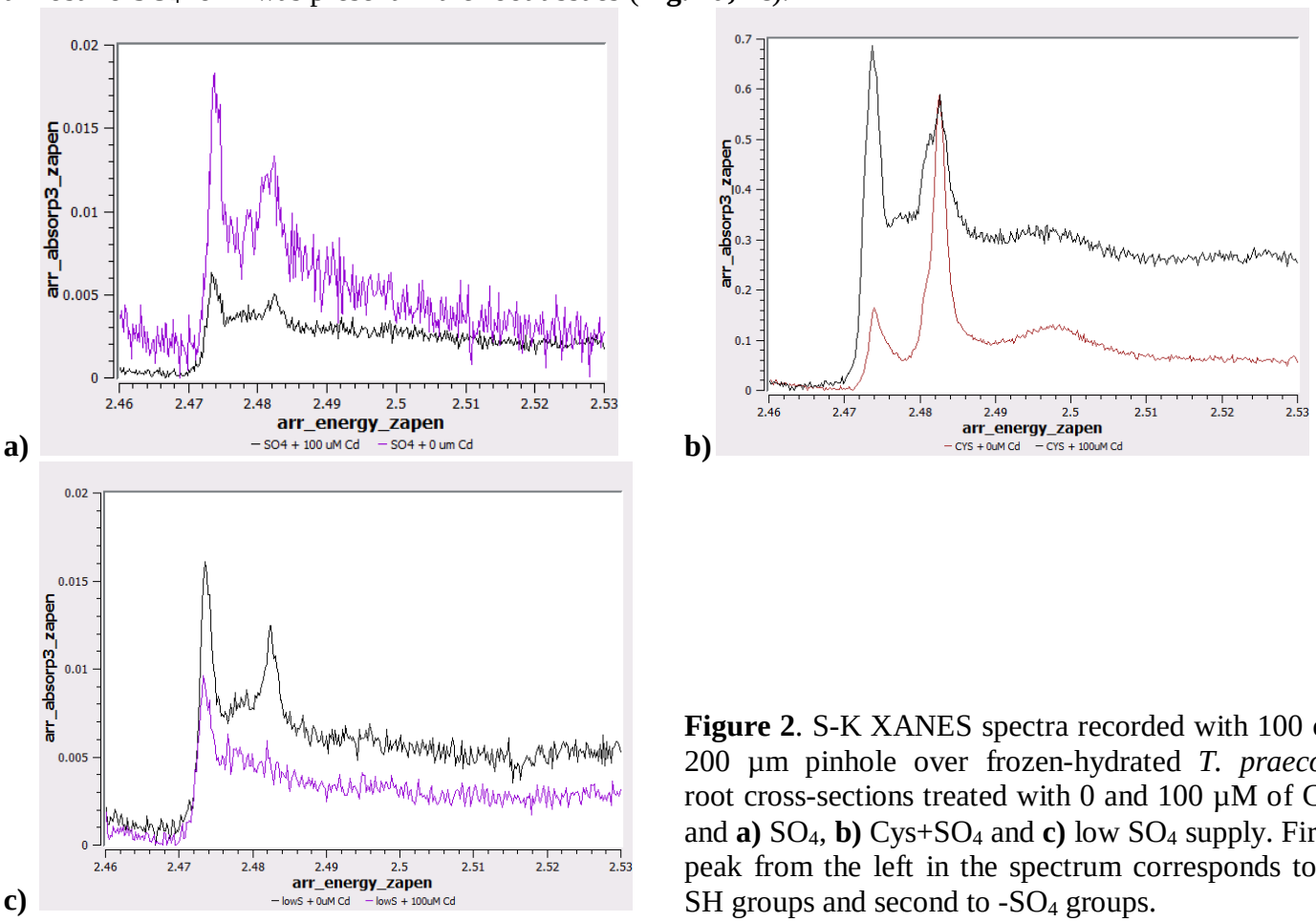

Figure 2. S-K XANES spectra recorded with 100 or 200 µm pinhole over frozen-hydrated *T. praecox* root cross-sections treated with 0 and 100 µM of Cd and **a)** SO_4, **b)** Cys+SO_4 and **c)** low SO_4 supply. First peak from the left in the spectrum corresponds to -SH groups and second to -SO_4 groups.

Treating plants with Cd resulted also in redistribution of -SH and -SO_4 groups in the roots as seen from S-K over-edge maps of roots fed with Cys. In both Cd treated (**Fig. 3a**) and non-treated roots (**Fig. 3b**) -SH groups were concentrated in central cylinder, however in Cd treated plants -SH groups were much more abundand in the root cortex, probably serving as sites for binding Cd.

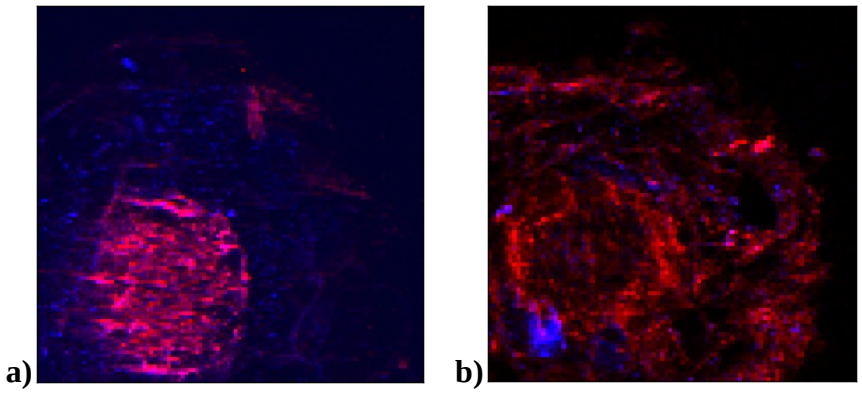

Figure 3. S-K over-edge maps of **a)** Cd treated and **b)** non-treated roots. Red channel corresponds to thiol (-SH) groups and blue channel to sulphate (-SO_4) groups.

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References

[1] K. Vogel-Mikuš *et al.*, Environ Pollut 133 (2005) 233-242.
[2] T. M. Vadas and B. A. Ahner, Environ Pollut 157 (2009) 2558-2563.
[3] M. M. Lasat *et al.*, Plant Physiol 118 (1998) 875-883.
[4] M. Wojcik *et al.*, Environ Exp Bot 53 (2005) 163-171.
[5] D. Ueno *et al.*, Plant Cell Physiol 49 (2008) 540-548.
[6] M. P. Isaure *et al.*, Spectrochim Acta B 61 (2006) 1242-1252.