ESRF	**Experiment title:** Speciation and distribution of mercury and selenium in roots of mycorrhizal and non-mycorrhizal plants	**Experiment number**: **SC-3448**
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Report:

Mercury (Hg) is one of the most toxic and hazardous metals found in environment, known to accumulate in food webs with a biomagnification pattern at successive trophic levels (Rimmer et al., 2010). Uptake, transport and transformations of Hg in plants depend on different soil properties as well as plants own metabolic processes. Inorganic Hg forms are usually less harmful than organic forms, partly because they bind strongly to soil components that reduce their availability and absorption. In contrast, organomercurials are highly toxic because of their hydrophobicity, which facilitates their movement across plant root cell membranes and accumulation in membrane bound organelles, inhibiting essential oxidative and photosynthetic pathways (Ruiz and Daniell, 2009) mainly through reactions of sulphydryl (-SH), phosphate and active ADP or ATP groups with cations, and replacement of essential ions, mainly major cations (Patra and Sharma, 2000). Hg has a high affinity for binding sulphur ligands such as glutathione (GSH) and phytochelatins, forming physiologically inactive complexes, protecting cytoplasmic processes which involve incorporation of metals into metalloproteins. In addition mycorrhizal fungi were proved to additionally restrict heavy metal accumulation and transport from the roots to the shoots (Kaldorf et al., 1999). Only little is known on the distribution of Hg in plant tissues and cells which may potentially impact Hg concentrations during food processing, therefore the aim of this study was to determine and compare localization, speciation and ligand environment of Hg in inoculated and non-inoculated plants.

Selenium (Se) is a trace element that can function as an essential nutrient for microorganisms, animals and humans (Fan et al., 2002). Although Se essentiality to higher plants is still under debate, the ability of some plants to accumulate and transform Se into bioactive compounds has important implication for human nutrition and health (Ellis and Salt, 2003). Uptake, transport and transformations of Se in plants also depend on different soil properties as well as form of Se (Terry et al., 2000). Toxicity occurs due to replacement of sulphur with selenium in amino acids resulting in incorrect folding of the protein and consequently non-functional proteins and enzymes (Germ and Stibilj, 2007). Since selenuim resembles chemical properties of sulphur and has high affinity to bind Hg, Se could play an important role in mercury detoxification (Mounicou et al., 2005). Therefore the knowledge on the behaviour of Hg and Se in plant organisms would

help us to characterize the mechanisms of Hg and Se toxicity and detoxification for the purpose of development of phytoremediation techniques for cleaning and stabilizing Hg with Se in Hg contaminated soils.

We expected that Hg will be distributed mainly in root epidermis and cortex and colocalized with sulphur. We expected Hg binding to thiol groups of proteins, low molecular weight thiols (cystein, glutathione) and phytochelatins to be the major detoxification mechanism. For Se we expected that the Se distribution as a result of Se (IV) uptake will be mainly localized in epidermal root cells and cell walls of cortical cells. The Se distribution as a result of Se (VI) uptake will lead to higher concentrations also in the symplast of the root cortex and leaf tissues.

For the study of Hg localization in maize roots the plants were grown in substrate amended with 50 mg/kg of $HgCl_2$ and preinoculated or not with arbuscular mycorrhizal strains isolated from mercury contaminated site. Both inoculated and non-inoculated plants were grown for two months under greenhouse conditions.

For the study of Se localization and speciation in plant tissues a hydroponic experiment was set. Maize and sunflower plants were grown in mineral nutrient solution with addition of 10 µM of K_2SeO_3 or 10 µM of K_2SeO_4. At the end of both experiments roots and leaves were cryo-fixed in propane cooled with liquid nitrogen, cut by cryo-microtome at – 25°C to 35 µm cross-section cuttings and freeze-dried. We also prepared some fresh hand cuttings just before the measurements. The freeze-dried or fresh tissue cuttings were mounted on specially designed holders in a sandwich between two ultralene foils. The X-ray beam delivered by the undulator was monochromatized by means of Si double crystal monochromator and focused to a 3.5 x 1.5 μm^2 probe using Kirkpatrick-Baez mirror focusing. The fluorescence emission of the sample was collected by the Si(Li) detector. The excitation energy for the scan was set to 13 keV (i.e above the Hg-L_{III} edge, 12285.59 eV and Se K-edge, 12665.6 eV) to record maps of Hg, Se and other elements below excitation energy like S, K, Ca, Mn, Fe, Cu and Zn. Se K-edge µ-XANES were recorded in different root and shoot parts, while we were not able to record Hg L_{III} – edge spectra of suficient quality due to too low Hg concentrations in the roots.

Hg was localized mainly in root cortex and endodermis, where colocalized with sulphur (**Fig. 1**). That confirms that Hg prefirentially binds with sulphur ligands as we expected.

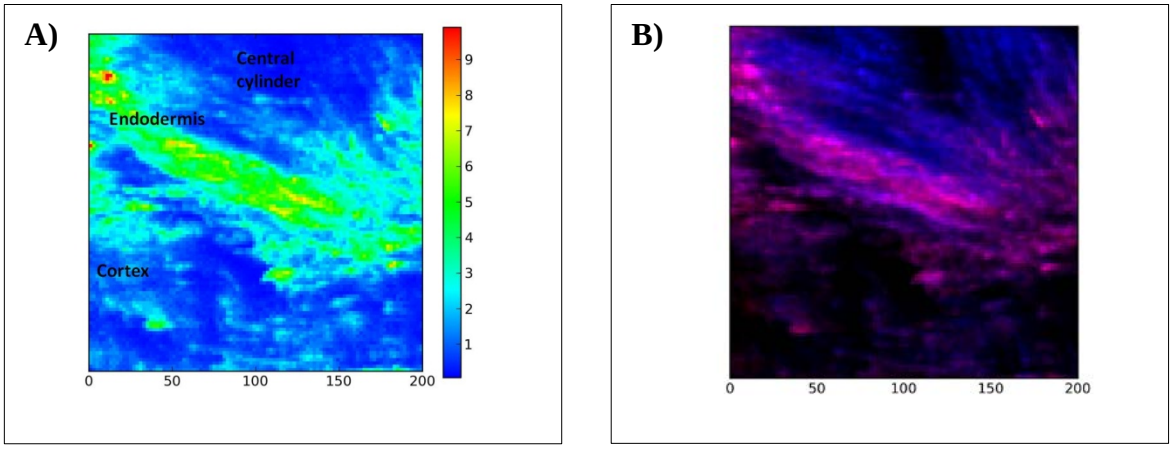

Figure 1. Mercury distribution in the maize non-inoculated root, showing retention of Hg in endodermis, where it is colocalized with sulphur, probably low molecular thiols and phytochelatins.

In Se^{4+} treated plants Se was maily localized in epidermal and subepidermal root tissues (Fig. 2), while in Se^{6+} treated plants higher concentrations were seen in root cortex (Fig 2). That indicates that Se^{6+} is more mobile when compared to Se^{4+}. The reason why Se^{4+} is less mobile may be because it is rapidly converted to organic forms of Se, which are retained in the roots (Zayed et al., 1998). Micro XANES spectra were recorded in different parts of the roots and shoots as well as Se-reference compounds. Different local environments of the Se cation result in different Se K-edge profiles. The detailed quantitative Se K-edge XANES analysis of all the measured samples is in progress. The results will be submitted for publication.

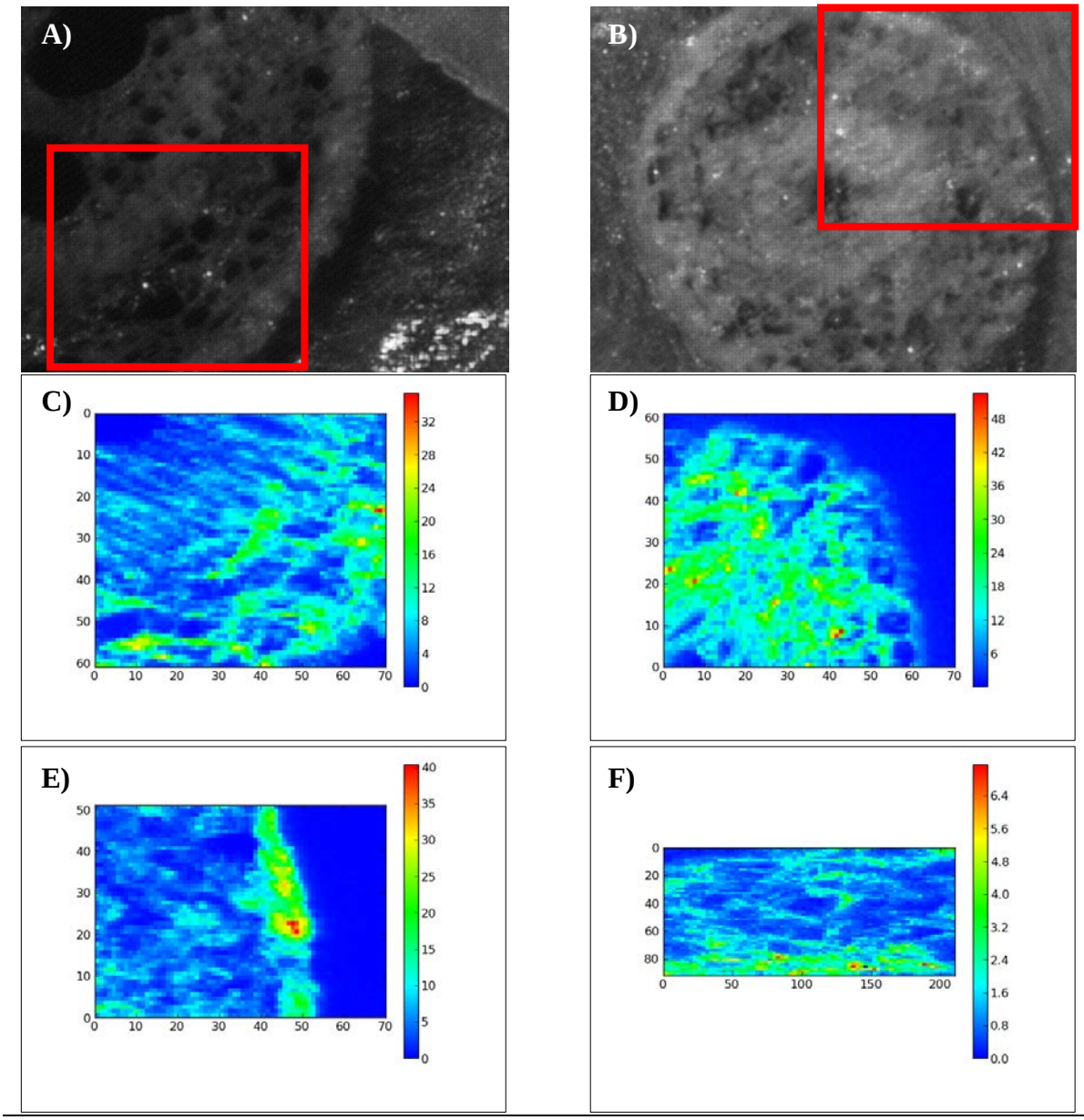

Figure 2. Videoimages of A) maize and B) sunflower roots. Se distribution in maize (C,D) and sunflower (E,F) roots treated with Se^{4+} (C,E) and Se^{6+} (D,F)

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