EUROPEAN SYNCHROTRON RADIATION FACILITY
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	Experiment title: Antimony Plant Uptake Mechanisms Unraveled by Speciation in Plant Roots and Shoots	**Experiment number**: EV-209
Beamline: BM23	**Date of experiment**: from: 14.11.2016 to: 21.11.2016	**Date of report**:
Shifts: 18	**Local contact(s)**: Sakura Pascarelli	*Received at ESRF:*

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Report:

Antimony (Sb) oxidation states, Sb(V) and Sb(III), show different toxicity[1] and can strongly affect plant uptake[2]. However the mechanisms of uptake and translocation within plants are still debated. Sb(V) is assumed to be taken up through the apoplastic pathway with the water stream or passively cross the electrochemical membrane gradient to enter the cell[3]. After this, there are two hypothetical translocation pathways 1) continuation along the apoplastic pathway were it must exist in an inorganic form ($Sb(OH)_6^-$) or be complexed by low molecular weight organics, 2) reduction to Sb(III) and chelation by glutathione (GSH), then storage in the vacuole as a means of detoxification. When present as $Sb(OH)_3$, Sb(III) probably passes through membranes via aquaglyceroporins[2]. If thiol compounds bind Sb(III) as a method of detoxification, this Sb can only be translocated after methylation of the complexes. Antimony concentrations in plant material are relatively low and solid speciation by synchrotron methods has not successfully been carried out before in this matrix. The aims of this study were a) establish if it is possible to analyse Sb speciation in plant material by XANES b) to establish the oxidation state of Sb in plant roots and shoots in plants exposed to different Sb oxidation states and species c) to identify which Sb species were present in this material by LCF of spectra of Sb reference compounds and from this elucidate the uptake and translocation pathways of Sb(V) and Sb(III) in plants.

Rye grass was treated with either Sb(III) ($Sb(OH)_3$), Sb(V) ($Sb(OH)_6^-$) or trimethylantimony(V) for eight days. Within the beamtime we analysed Sb in rye grass and reference antimony compounds by XANES using a He cryostat due to the low plant concentrations. We used freeze-dried plant material in order to ensure high enough Sb concentrations to obtain a good signal to noise ratio. After harvesting, the plants were quickly frozen in liquid nitrogen and freeze dried. The dried material was homogenised using a ball mill in a glove

box under anerobic conditions and then pressed into pellets which were mounted on the He cryostat holder of the beamline. The roots of the Sb(III) treated plants, which had the highest concentrations of all treatments, were also analysed under frozen hydrated conditions to investigate if the freeze drying process led to any change in Sb speciation. The plants were harvested and the roots were rapidly frozen and homogenised in a mortar in liquid nitrogen before pressing into pellets and transfer to the beamline cryostat.

The XANES Sb-K edge (30,491 eV) spectra were measured in fluorescence or tranmission detection mode depending on the concentration of Sb in the sample at BM23 using a double crystal Si(311) monochromator. A thirteeen segment germanium solid state detector was used to collect the fluorescence signal and an antimony reference foil was used for spectra normalization. Spectra of between two and sixteen identical runs were superimposed to improve the signal-to-noise-ratio. The spectra where analysed with the Athena software package[4]. The relative amount of antimony compounds in the plants were analysed with the linear combination fit method using the reference antimony compounds[4].

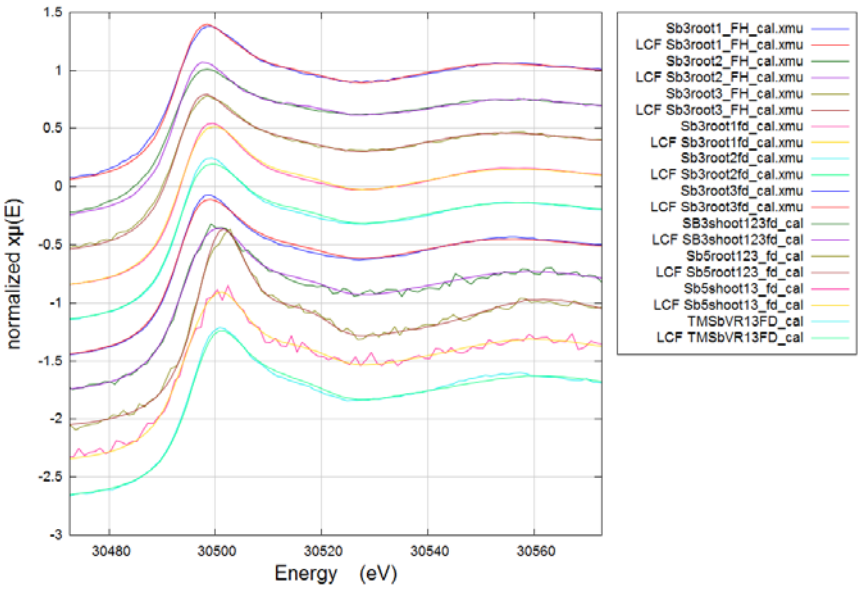

Figure 1: Sb K-edge XANES spectrum of roots and shoots of Sb treated plants with LCF of reference compounds.

All plant samples were analysable except for the shoots of the trimethylantimony treatment where the concentrations proved to be too low even with repeat scans. The process of freeze drying resulted in only a slight shift in oxidation state of antimony in the samples. After combining replicate scans of the same samples and in some cases of replicate samples it was possible to carry out linear combination fitting for all plant samples using the antimony reference compounds and achieve a reasonable fit.

The results have been published in : Fate and chemical speciation of antimony (Sb) during uptake, translocation and storage by rye grass using XANES spectroscopy, Ji Y. et al., Environmental Pollution, 2017, https://doi.org/10.1016/j.envpol.2017.08.105.

References

[1] C. Wei, Z. Gea, W. Chu, R. Feng, Speciation of antimony and arsenic in the soils and plants in an old antimony mine, Environ. Exp. Bot., 109 (2015) 31-39.
[2] X.M. Wan, S. Tandy, K. Hockmann, R. Schulin, Changes in Sb speciation with waterlogging of shooting range soils and impacts on plant uptake, Environ. Pollut., 172 (2013) 53-60.
[3] M. Tschan, B.H. Robinson, M. Nodari, R. Schulin, Antimony uptake by different plant species from nutrient solution, agar and soil, Environ. Chem., 6 (2009) 144-152.
[4] B. Ravel, M. Newville, ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT, Journal of Synchrotron Radiation, 12 (2005) 537-541.