ESRF	**Experiment title:** Study of Arsenic uptake by gravepines inoculated or not with soil bacteria with ability for phytoremediation	**Experiment number**: 08-01-1040
Beamline: BM08	**Date of experiment**: from: 04/06/2018 to: 08/06/2018	**Date of report**: 17/07/2018
Shifts: 9	**Local contact(s)**: Francesco d'Acapito	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

BARALDI Rita*, CNR-IBIMET Bologna

PETROSELLI Chiara*, Università degli Studi di Perugia Dipartimento di Chimica, Biologia e Biotecnologie

NERI Luisa, CNR-IBIMET Bologna

BOTTINI Ruben, Universidad Nacional de Cuyo Facultad de Ciencias Agrarias IBAM

PICCOLI Patricia, Universidad Nacional de Cuyo Facultad de Ciencias Agrarias IBAM

Report:

Scientific Background

Arsenic and its inorganic compounds are classified as carcinogenic and its organic compounds metabolized by humans as possibly carcinogenic. The main factors affecting As in soils are rock composition and human activities (mining, smelting, combustion of fossil fuels, pesticides and herbicide application). Literature data suggest an average value of 20 mg kg^{-1} for Italian soils (range 1.8-60 mg kg^{-1}) but widespread hot spots occur in volcanic and mining areas. In Northeaster Italy (the Brenta Plain) value of As is higher (36 mg kg^{-1}) than the regulatory threshold (20mg kg^{-1}) while in West central Italy (Mt Amiata mining district) significant contamination with As was also found in river particulate matter. In Río Jáchal basin, San Juan, Argentina, a high concentration of As in soils exceeding the limits established by Argentina regulations (20 ppm) has been found. The highest As concentration in soil was found in the Iglesia distric (100-125 mg g^{-1} dry soil) where As content in grape berries varied between 100 to 200-fold those established by the CAA (Argentine Food Code; 100 ppb for vegetables), close to a concentration threshold considered phytotoxic. On the other hand, the Huaco River, located in the same region but originates from rainwater shows low As. In grapevine, it has been found As concentrations in leaves ranging from 60 to 410 µg kg^{-1}DW, although in berries and wine a concentration <0.5 to 17 µg L^{-1} is below the limit

of 200 µg kg^{-1}Dry Weight (DW). The potential for good quality wines is not however fully exploited because of the treat of As presence in both grapes and their product (wine). Therefore, in order to attend to both, the people´s health and quality of the product, evaluation of practices of phytoremediation and/or prevention in contamination are mandatory. Strains of Plant Growth Promoting Rhizobacteria (PGPR) has been isolated from vineyard soils in Argentina, and some of these PGPR showed high capacity to tolerate As when cultivated *in vitro*, diminishing its concentration in organs of inoculated plants. However, the mechanisms involved in such alleviation are not well understood. Besides, to use phytoremediation effectively and efficiently, it is important to understand the biochemical pathways and mechanisms that operate to translocate As species from the soil to the shoots. Our hypothesis is that bacteria modify the chemical species of As, such as thiol-rich compounds, therefore the plant membranes render unable to incorporate it or diminish the relation of the more toxic and bio-available form As^{III} and the oxidized form As^{V} so the As state undergo unavailable for uptake in the rhizosphere.

Experimental details, measurement strategy

XANES and EXAFS spectra at the As k-edge (11867 eV) have been collected at the CRG-LISA beamline (BM08). Fluorescence spectra have been recorded at room temperature in moderate vacuum conditions over the samples pellets. The new experimental setup with a Si(111) crystal monochromator and an SDD multi-element detector array (ARDESIA, Bellotti, G., Butt, A.D., Carminati, M., Fiorini, C., Bombelli, L., Borghi, G., Piemonte, C., Zorzi, N., Balerna, A. The ARDESIA Detection Module: a 4-Channel Array of SDDs for Mcps X-Ray Spectroscopy in Synchrotron Radiation Applications (2018) IEEE Transactions on Nuclear Science, Article in Press., DOI: 10.1109/TNS.2018.2838673, 2018.) determined a significant improvement of the experimental conditions, especially for diluted samples. The spatially extended beam (about 2 x 5 mm) ensured representative bulk analyses and not single particle ones. Four scans per sample have been averaged, in order to improve the signal-to-noise ratio and beam damage can be excluded by comparing the first and the last spectrum of each series. The transmission spectrum of a reference sample (metallic As foil) has been recorded at the same time of each sample scan in order to provide a reliable internal energy calibration.

Samples details

Vitis vinifera L. Cv. Malbec plantlets have been grown *in vitro* in a MS medium and kept in a growth chamber at 24°C with a photoperiod of 6 h of light provided by incandescent lamps. Two treatment, consisting of 6 plantlets each, were compared: Control (only Arsenic) and PGPR (Arsenic+PGPR). PGPR were isolated from rhizosphere and vine roots, in Jáchal, San Juan, and grown in liquid LB medium for 48 h. Plantlets were inoculated, or not, by adding 20 µL of bacterial culture. After 7 days, 150 µM of AsIII was added to the MS medium of both control and treated plantlets. After 40 days of treatment, the plants have been dissected in roots, leaves and shoots, oven-dried and ground to powder with liquid nitrogen. Six samples have been prepared, three corresponding to the roots, shoots and leaves of Control plantlets and three corresponding to PGPR inoculated plantlets. Since the overall quantity of the samples was low, the samples have been pressed into small pellets (d=5mm) using pure cellulose as a binder. The correct amount of sample and cellulose for fluorescence-suitable

pellets has been calculated with the XafsMass software. All the analysed samples will undergo quantitative As concentration analyses.

Results obtained

During the experimental session at the CRG-LISA beamline, we were able to measure XANES and EXAFS spectra of six selected samples: roots, shoots and leaves of As-treated plants and the same organs for control and PGPR-inoculated plantlets. Preliminary fluorescence analyses allowed us to estimate arsenic concentrations in samples and noticeable differences between inoculated and non-inoculated plants emerged both in the overall quantities and in the distribution between the different plant organs. These observations need to be verified by means of quantitative As concentration analyses (e.g. ICP-MS). From the XANES spectrum, differences in the As(III)-As(V) proportion for PGPR-inoculated plants with respect to non-inoculated ones are evident. The comparison of the samples' spectra with the measured standard compounds' ones will allow a more quantitative determination of the As(III)-As(V) proportion and, eventually, a clarification on the presence of lower oxidation states. EXAFS spectra have been collected up to k=12 and 4 scan per sample have been averaged in order to obtain quantitatively analysable spectra. The analysis of the oscillating part of the spectrum, which is currently in progress, will allow to determine the nature of the neighbouring atoms, the coordination and, hopefully, to identify the As accumulation mechanism.

Figure 1 – Fluorescence spectra (lefthand panel) and XANES spectra (righthand panel) of leaves of PGPR- inoculated or non-inoculated plants grown in Arsenic enriched culture medium.