ESRF	**Experiment title:** The origin and fate of organic selenium in seleniferous soil of Punjab, India: insight from selenium speciation in soils and plants	**Experiment number**: A16-1-818
Beamline: BM16	**Date of experiment**: from: 04th July 2023 to: 10th July 2023	**Date of report**: 09 September 2023
Shifts: 18	**Local contact(s)**: Dr. Olivier Proux	*Received at ESRF:*

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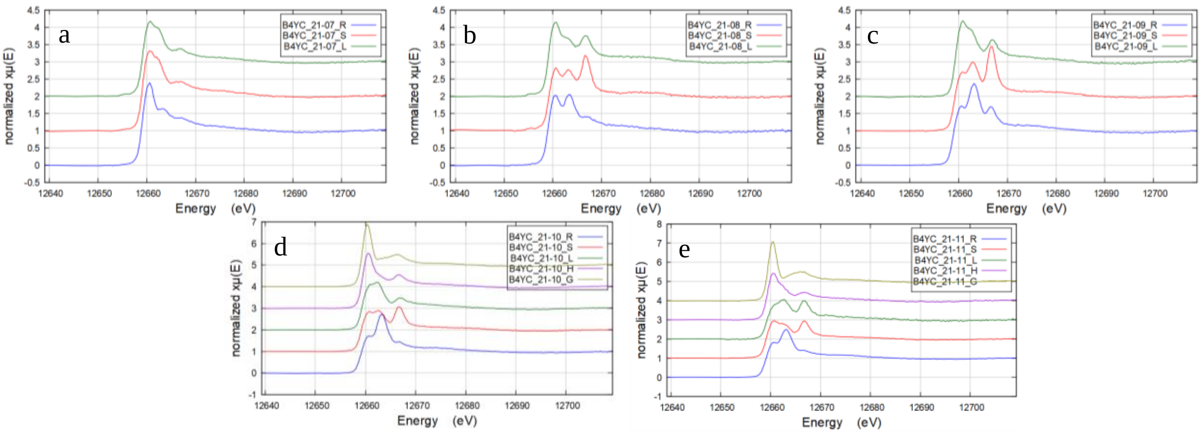

Fig. 1: Normalized Se K-edge XANES spectra of different parts of the rice plants collected monthly from a seleniferous land. Sample ID represents sampling site (B4YC)_sampling time (year-month)_plant part (root as R, stem as S, leaf as L, husk as H, and grain as G).

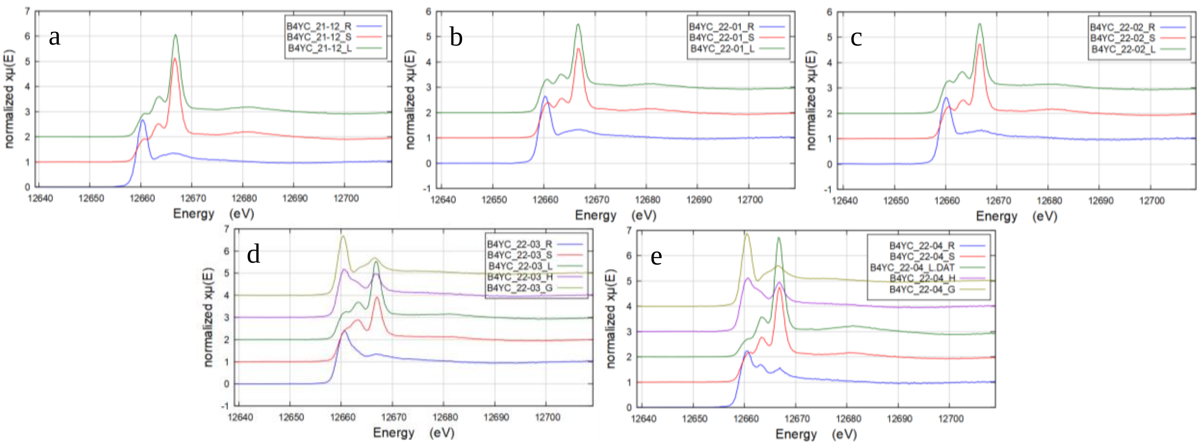

Fig. 2: Normalized Se K-edge XANES spectra of different parts of the wheat plants collected monthly from a seleniferous land. Sample ID represents sampling site (B4YC)_sampling time (year-month)_plant part (root as R, stem as S, leaf as L, husk as H, and grain as G).

Report:

Background of the experiment: In our first experiment at the BM16 beamline (EV-460), we determined the dynamics of selenium (Se) speciation in the seleniferous soil of Punjab, India, in relation to different agricultural activities performed on the land. Topsoil (0 to 5 cm) samples, collected monthly over an entire season of the rice-wheat crop rotation (11 months) from an agriculture plot with a history of 4 years of crop-residue (in the form of rice straw) incorporation into the soil, were measured for the Se K-edge bulk XANES at the BM-16 beamline. Results showed that although Se speciation in the soil shows considerable temporal variability, closely linked to the irrigation pattern practiced for rice and wheat cultivation, organic Se generally dominates the Se speciation in the soil. Therefore, the second experiment (A16-1-818) aimed to investigate two fundamental questions. First, what is the source of organic Se in this soil? Second, is the organic Se bioavailable for plant uptake?

Experiment at the beamline: We started by investigating the second question and completed it within the allocated beamtime of 18 shifts. We have collected Se K-edge bulk XANES data for different parts (viz. root, stem, leaf, husk, and grain) of the rice and wheat plants (total 42 samples), sampled monthly from the same agriculture plot from where soil samples were measured in the first experiment. However, we could not analyze additional soil samples within the allocated beamtime, as proposed in the experiment proposal, to investigate the origin of the organic Se in this soil.

Data processing and results obtained so far: The energy calibrated, merged, and normalized Se K-edge spectra for rice and wheat plant parts are displayed in Fig. 1 and 2. Three peaks in the energy position of organic Se ((~12660 eV), selenite (~12663 eV), and selenate (~12666 eV) dominate the spectra of the measured samples. For rice, at the initial stage of the cultivation, Se speciation in root, stem, and leaf was dominated by organic Se (Fig. 1a), suggesting that Se was probably taken from the soil predominantly as organic Se. With the progress of the cultivation, organic Se was metabolically transformed into inorganic Se (viz. selenite and selenate). Despite such species transformation, however, organic Se could be unloaded into the grain substantially (Fig. 1). For wheat, Se was present in the root almost exclusively as the organic Se for the major part of the cultivation (Fig. 2), again highlighting that Se was taken from the soil in the form of organic Se. This finding emphasizes the importance of determining the origin of organic Se in this soil, which could not be determined in this experiment due to the limited availability of the beamtime. Therefore, a proposal for further beamtime at the BM16 beamline will be submitted for additional soil analysis.

However, unlike rice plants, for wheat, Se speciation in the stem and leaf was dominated by the inorganic Se, especially selenate, from the beginning of the cultivation (Fig. 2). Also, the inorganic Se content in the husk and grain was higher in wheat than rice. There can be two hypotheses to explain the dominance of inorganic Se in the stem and leaf of wheat plants. First, the metabolic transformation of organic Se into inorganic Se is much faster in wheat than in rice. Second, inorganic Se species could also be taken up from the soil with organic Se and translocated to the shoot (stem and leaf) faster than organic Se, resulting in overall higher Se uptake, accumulation, and toxicities in wheat plants. The relatively more oxic conditions in the soil during wheat cultivation could lead to the formation of selenate, the highest mobile Se species in the environment. However, our first experiment (EV-460) at the BM16 Beamline could not identify selenate in this soil, even during the stage of wheat cultivation. Nevertheless, this experiment clearly showed that the distribution of Se species in this soil can vary considerably at the micrometer scale. There is a possibility that selenate can be concentrated spatially in the soil but in trace amounts at the bulk level, which could not be determined by the bulk XANES analysis. Therefore, determining the microscale heterogeneity in Se speciation in this soil is warranted to understand the Se cycling completely at the soil-plant interface. Also, controlled experiments in the laboratory to understand the uptake, metabolism, and translocation of organic Se in rice and wheat plants are required to explain the field data.

Currently, we are identifying the specific Se species representative of the organic Se, selenite, and selenate that may be present in these samples. With spectra of the identified species, linear combination fitting of the sample spectra will be performed to quantify the distribution and variability of these species in the different parts of the rice and wheat plants over the season of the rice-wheat crop rotation.