EUROPEAN SYNCHROTRON RADIATION FACILITY
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	Experiment title: **Biotransformation of mercury and selenium in edible mushrooms *Boletus* sp. - indication for bioavailability and food safety**	**Experiment number**: EV-236
Beamline: BM30b	**Date of experiment**: from: 9.2.2017 to: 14.2.2017	**Date of report**: 1.9.2017
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Report:

Background and aims

Wild growing edible mushrooms are regularly consumed in many European countries. The fruiting bodies of *Boletus edulis* are considered "the wild mushroom *par excellence*" in Italian, French and German cuisine for their aromatic taste and versatility. Yet *Boletus* species accumulate relatively large amounts Hg, but also contain relatively high amounts of Se [1]. By our preliminary results *B. edulis* in Slovenia accumulates up to 10 μg g^{-1} dry mass of Hg in neutral reference area, while in the vicinity of Idrija Hg mine, Hg conc. of up to 170 μg g^{-1} were detected [2]. For Se, conc. from 20-70 μg g^{-1} are reported [3].

Recently we showed that arbuscular mycorrhizal fungi are able to bind Hg to 4 S ligands while in plants Hg-2S complexes are mainly found [4]. The aims of this study were a) to determine speciation and ligand environment of Hg and Se by EXAFS and XANES in *Boletus* sp. fruiting bodies collected in natural environment, b) to follow the processes of biotransformation of Hg and Se in *in vitro* tissue culture of *B. edulis* including their uptake and accumulation, and c) to link the Hg and Se coordination to their bioavailability and toxicity by feeding the mushrooms to the slugs (*Arion* spp.), monitoring toxicity-sensitive biochemical parameters in snail hepatopancreas, namely the level of lipid peroxidation via MDA test.

Experimental set-up

The experiment was divided into 3 parts:

1) The mushrooms (*B. edulis*) were collected at various polluted and non-polluted areas in Slovenia. Parts of caps and stipes were used for the analysis of the total Hg and Se contents by XRF and ICP-MS, while the other parts were frozen, ground and stored in frozen-hydrated state (pellets) or freeze-dried in order to pre-concentrate Hg, for XAS measurements in cryo conditions at 4K using He cryostat.

2) Hg uptake in *B. edulis* was studied with and without added Se, grown in tissue cultures. The fungi were grown in Petri dishes on potato dextrose agar supplemented with Hg in different forms and concentrations of (0, 1, 10, 50, 100 mg kg^{-1} of HgS and $HgCl_2$) and without or with added Se (10 mg kg^{-1}). Fungal growth and stress parameters were monitored (biomass of mycelium, MDA levels and polyphenol content). Hg-L3 EXAFS was measured on 100 mg Hg kg^{-1} treated fungi.
3) Mushrooms collected in the natural environment and those grown in *in vitro* culture (0, 100 mg kg^{-1} of HgS and $HgCl_2$, with and without added Se - 6 treatments) were fed to the slug snails. Hg-L3 and Se-K EXAFS ligand environment was determined in snail hepatopancreas tissues, while in muscles Hg concentrations were too low.

XAS analysis

XAS spectra were measured at BM30B beamline in fluorescence detection mode (using large area Ge detector), because of low contents of Hg and Se in the samples. In addition, EXAFS/ XANES spectra of some reference Hg [4] and Se compounds with known valence state and ligands were measured in transmission mode and added to our database of metal-ligand compounds. The reference samples were prepared as homogenous self-standing pellets with total absorption thickness ~ 2 above the edge. Spectra were analysed with IFEFFIT program package, exploiting LCA and PCA analysis for XANES spectra and multiedge parallel fitting of EXAFS spectra [4,5].

Results

Boletus edulis and *Albatrellus pes-caprea* contained 80 and 40 mg Hg kg^{-1} and 20 and 500 mg Se kg^{-1}. Hg-L3-EXAFS analysis (Figure 1) showed that Hg is coordinated mainly to S and N ligands in *B. edulis*, while Se ligands were also present in a Se hyperaccumulator *A. pes caprae*. Hg-2S and Hg-4S coordination was found in *B. edulis*, while only Hg-2S coordination was seen in *A. pes caprae*. *Boletus* caps contained higher proportion of Hg-4S coordination than the stalks. Hg-4S coordination is typical of Hg binding to metallothioneins. In vitro cultivated fungi that grew in the presence of $HgCl_2$ contained the highest proportion (69%) of Hg coordinated to 4S, indicating different fungal behaviour with different Hg compounds. In the natural environment Hg is present mainly as poorly soluble HgS or Hg^0, while $HgCl_2$ easily dissociates to Hg^{2+} ions. Hg^{2+} ions induce metallothioneins synthesis in fungal tissues in order to chelate and detoxify Hg^{2+} ions.
Bioavailability studies, as assessed by a slug snail model showed significantly lower Hg bioavailability in *A. pes caprae*, where the majority of Hg was bound to Se (84% of Hg-Se-C), indicating that Hg-Se-C complexes are much less soluble that Hg-2S and Hg-4S.

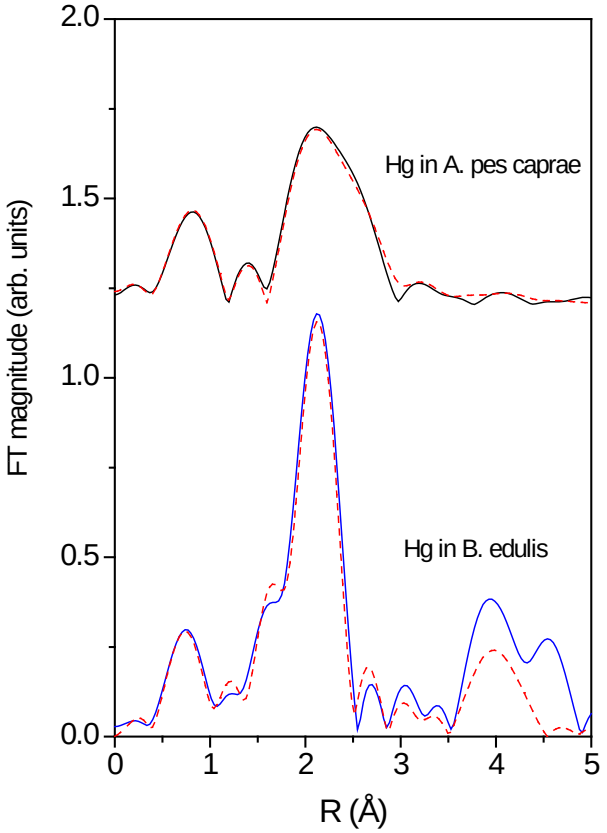

Figure 1. Fourier transform magnitude of k^2-weighted Hg-L_3 EXAFS spectra recorded on *B.edulis* and *A. pes caprae*. Experiment – (solid line); best fit EXAFS model in the R range from 1.2–4.0 Å – (dashed line).

References

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