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THE EFFECT OF SELENIUM ON MERCURY TRANSPORT ALONG THE FOOD CHAIN

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ABSTRACT

More than 500 years of mercury (Hg) production in Idrija (Slovenia) resulted in a considerable pollution of Idrija region with Hg. Although the mine is closed for more than 20 years, the total soil concentration of Hg may still reach up to several hundred mgkg⁻¹ dry weight in local gardens and more than thousand in other urban regions. Hg in soil undergoes different chemical transformations and in some forms it may enter plants and higher trophic levels in food chains, also with biomagnification pattern. The local population is, besides air and dust, thus exposed to mercury also via consumption of locally produced food. Several studies showed that the increased level of selenium in soil may reduce the uptake of mercury in plants but very few include other trophic levels in a food chain as well. In our pilot study we followed an impact of Se on Hg transport from soil to plants (*Lactuca sativa*) and further to soil dwelling animals (*Porcellio scaber*). Lettuce was planted in a contaminated soil from Idrija and in soil with added HgCl₂. The leaves of half of the plants were sprayed with Se solution (5 μg L⁻¹) three and five weeks after planting. After six weeks plants were analyzed for Hg and Se and offered as food to terrestrial isopods for two weeks. Our preliminary results revealed that foliar treatment of plants with Se may affect Hg accumulation in plants and therefore further transport of Hg across the food chain.

Keywords: *mercury, selenium, isopods, Lactuca Sativa, food chain.*

INTRODUCTION

Mercury exists in different forms: inorganic mercury, which includes elemental mercury, mercurous and mercury salts; and organic mercury, where mercury is bound to a methyl, ethyl, phenyl, or similar groups (Bernhoft 2012). Toxicity of mercury to wildlife and humans varies with the form, the dose and the rate of exposure (Wolfe et al., 1998; Tchounwou et al., 2003; Bernhoft, 2012). Elemental

mercury is usually inhaled while other forms are usually ingested. Organic (methyl) mercury, which is known as the most toxic form, can be inhaled or absorbed through skin as well. Elemental and methyl mercury react with sulfhydryl groups and sulphur-containing amino acids, therefore potentially interfering with the function of any cellular or subcellular structure. In contrast, mercurous and mercury salts are poorly soluble and poorly absorbed and cause damage predominantly to the gastrointestinal tract or kidney (Bernhoft 2012).

In the last decades many large Hg mines had been abandoned worldwide because of lower demand for Hg. The primary concern about former Hg mines is the accumulation of Hg in soil and sediments, its transfer and biomagnification over food webs and the conversion from inorganic to organic Hg during these paths. Near the former world's second largest mercury mine in Idrija, Hg concentrations still range from 7 up to 1550 mg kg⁻¹ in urban soil and from 22-320 mg kg⁻¹ in garden soil (Bavec and Gosar 2016). There are two major exposure pathways for the local population: exposure to atmospheric Hg and to Hg in food. In 2003, the estimation for daily intake of Hg was 0.05-0.1 µg Hg per kg body weight by inhalation and 0.66 µg Hg per kg of body weight via food (other than fish)(Horvat et al., 2003). It would therefore be very advisable to reduce Hg uptake via foodstuff, predominantly of locally produced vegetables. Due to the difficult economic situation in the region and to the desire of the population to produce organic food locally, it is impossible to prohibit the consumption of locally produced vegetables and other ways will have to be found. One possibility is to reduce the uptake of Hg by plants as well to reduce the bioavailability of Hg in plants to consumers. In the last decades several studies showed the antagonistic action of Se in soil on the uptake and translocation of Hg in plants (Shanker et al., 1996a,b; Thangavel et al., 1999; Mounicou et al., 2006; Zhang et al., 2012). Mercury has a high affinity to bind with Se and form insoluble mercury selenides, which can prevent negative effects of mercury in animals (rev in Raymond et al 2004). Selenium is also an important essential micronutrient in humans and other animals (Rayman 2000). It has a structural and enzymatic role, among others it acts as an antioxidant and catalyst for the production of active thyroid hormone and is important for the proper functioning of the immune system. At high concentrations, however, it causes toxicity (Yang et al 1983). By adequate application of Se to plants we might therefore decrease the transport of Hg across the food web as well as enrich the food with Se.

The aim of this study was a) to get some information about the impact of controlled foliar application of Se on the uptake and translocation of Hg in *Lactucasativa* from spiked and natural Hg-contaminated soil and b) to evaluate the impact of Se on Hg transport from lettuce to terrestrial isopods (the consumers), the next trophic level.

MATERIALS AND METHODS

Plant experiment

Three weeksold lettuce plants (*Lactucasativa* cv. Exquise) were planted into pre-prepared substrate - non-contaminated mixture of pot and field soil (C), mixture of

pot and field soil spiked with $50 \mu\text{g g}^{-1}$ of HgCl_2 (Hg 50) and garden soil from Idrija (Idrija). For each treatment 10 plats were planted. Non-contaminated soil was prepared by mixing an organic potting substrate (Biobrazda, Slovenia) with sieved soil collected in the field near the Biotechnical Faculty, Ljubljana Slovenia (ratio 1:1). The first half of the mixture was left untreated, while the second half was spiked with $50 \mu\text{g g}^{-1}$ of HgCl_2 (Merck, Germany) in a form of solution and mixed thoroughly. The spiked soil was left in a closed plastic bag for five days to achieve equilibrium. Hg concentration was below the limit of detection of ICP-MS ($<0.1 \mu\text{g g}^{-1}$), pH=7, organic matter=13%. Soil collected in the garden in Idrija that was naturally contaminated with Hg, contained $140 \pm 5 \mu\text{g g}^{-1}$ of total Hg as determined by XRF (Ne emer et al., 2008), pH=6, organic matter =10%.

Lettuce plants were grown in climate chambers (24°C , 60% relative humidity, 16/8 day/night photoperiod, photon flux = $300 \mu\text{mol m}^{-2} \text{s}^{-2}$) for six weeks. After three and five weeks of growth the plants were sprayed with Se solution (selenate as K_2SeO_4 (Alfa Aesar), $5 \mu\text{g L}^{-1}$). Estimated total dose of applied Se was $1 \mu\text{g g}^{-1}$ fresh weight.

At the end of the experiment the plants were harvested, the shoots were detached from the roots and the roots and shoots were thoroughly washed in tap and distilled water. Plant material was then packed in Al foil, rapidly frozen in liquid nitrogen and freeze-dried (freeze-drier 2-4Alpha-Christ) for one week. After freeze drying the dry plant biomass was determined. Aliquots of 100 mg were used for Hg and Se analysis by ICP-MS.

Animal experiment

The plant material was ground in a mortar with liquid nitrogen and pellets ($\phi = 1 \text{ cm}$) were pressed from the root:shoot mixture (1:1) using a pellet die and a hydraulic press to feed the animals *ad libitum*. The pellets contained on average 3.92, 3.12, 0.56 and $0.23 \mu\text{g Hg g}^{-1}$ dry food in 50Hg, 50Hg+Se, Idrija and Idrija+Se treatments, respectively.

Twenty animals of laboratory bred terrestrial isopods (*Porcellioscaber*) (weight 30-50 mg) were selected per treatment and put individually into Petri dishes ($\phi = 14 \text{ cm}$) on moist filter paper. Food pellets were offered in small plastic dishes ($\phi = 5 \text{ cm}$) that separated food from moist filter paper and were changed every 5 days. Animals were kept for 14 days in climate chambers at 20°C , 80 % relative humidity and 12/12 day/ night photoperiod. At the end of the experiment the animals were fed for three days with non-contaminated food to clean the gut. Afterwards the animals were frozen in liquid nitrogen, freeze-dried (freeze-drier 2-4Alpha-Christ) for three days, and weighted.

Hg and Se analysis with ICP-MS

Hg and Se concentrations in plants (4 per treatment) and Hg concentrations in animals (10 per treatment) were determined by ICP-MS (Agilent 7500ce, Palo Alto, CA USA) after microwave assisted digestion (MarsXPress, CEM, 15 min ramp to 180°C , 30 min hold at 180°C , 1000 W) in concentrated HNO_3 and stabilization of

digests by HCl (Debeljak et al., 2013). The results were validated by measuring standard reference materials (BCR/CRM-061 Aquatic plant, NIST; ERM Tuna fish, Sigma Aldrich).

Statistical analysis

ANOVA and Duncan's post hoc test were performed by Statistica Statsoft 7.0 software.

RESULTS AND DISCUSSION

Plant growth parameters

The shoot biomass was the most affected by Hg treatment and significantly decreased in 50 Hg and 50 Hg+Se plants (Fig. 1). Although the roots were the organs that were directly exposed to Hg, their biomass in the 50 Hg treatment even increased (Fig. 1). The total plant biomass (roots + shoots) was the highest in C+Se treatment and the lowest in 50 Hg+Se treatment.

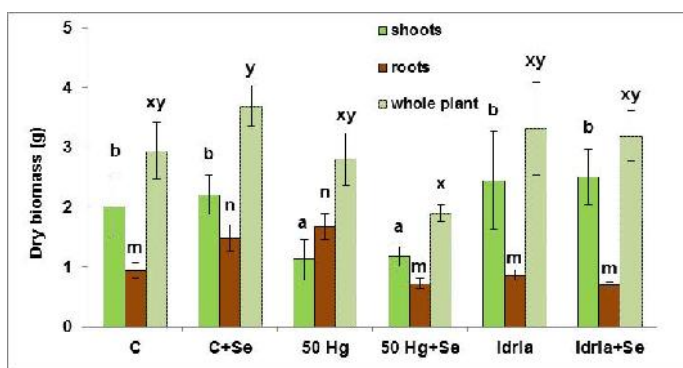


Figure 1. Dry biomass of roots, shoots and whole lettuce plants (*Lactucasativa*) ($\text{avr} \pm \text{SE}$, $n=4$) that were grown in mercury contaminated soil with or without foliar spraying with selenium solution ($5 \mu\text{g L}^{-1}$). Different letters above the columns represent statistically significant differences (a, b - shoots; m, n - roots; x, y - whole plant), Duncan's post hoc test, $p < 0.05$.

In non-stressed plants Se may act as a growth promoter and increase plant growth (White, 2016), while in stress conditions the effects of Se spraying may induce adverse effects (Sors et al., 2005). The plants grown in the substrate collected in Idrija were not affected by Hg or by Se treatment. Although garden soil from Idrija contained higher total amounts of Hg than in the 50 Hg treatment, Hg was more bioavailable and more toxic in the latter substrate, where Hg was present as HgCl_2 (ionic Hg^{2+} form). In the soil collected in Idrija, however, Hg is present mainly as cinnabar (HgS) (more than 80%) or metal mercury (Hg^0) as shown by fractionation studies (Kocman et al., 2004). It has to be also emphasized that the used substrates had different pH, amounts of organic matter and different element composition (data not shown), which may affect the plant growth from the perspective of mineral nutrition, as well as synergistic/antagonistic effects of different ions present in the substrate (e.g. Ca, Fe) (Sarwar et al., 2010).

Hg and Se concentrations in plant tissues

Hg accumulated mainly in plant roots with concentrations that were on average up to seven times higher in the roots compared to the shoots (Fig. 2a). This partitioning of mercury was observed also in other plants, such as *Rumexinduratus*, *Marrubium vulgare*, *Medicagosativa* and maize (Carrasco-Gil et al., 2013; Debeljak et al., 2013; Moreno-Jiménez et al., 2006). The highest Hg concentrations were seen in the 50 Hg treatment. Foliar application of Se decreased Hg concentrations in the roots of both the 50 Hg and Idrija treatments. In the shoots, however, this trend was not seen in the 50 Hg treatment, while in Idrija treatment the Hg concentration in the plants sprayed with Se decreased (Fig. 2a). Shoot Hg concentration reflects the Hg that is transported from the roots to the shoots, as well as volatile Hg that vaporizes from the contaminated substrate and adsorbs on the leaf surface or enters the leaves through the stomata (Moreno-Jiménez et al., 2006). Therefore it is very hard to determine the portion of Hg transported from the root to shoot, especially at such low concentrations, where any contamination from dust or soil particles may significantly influence the final values.

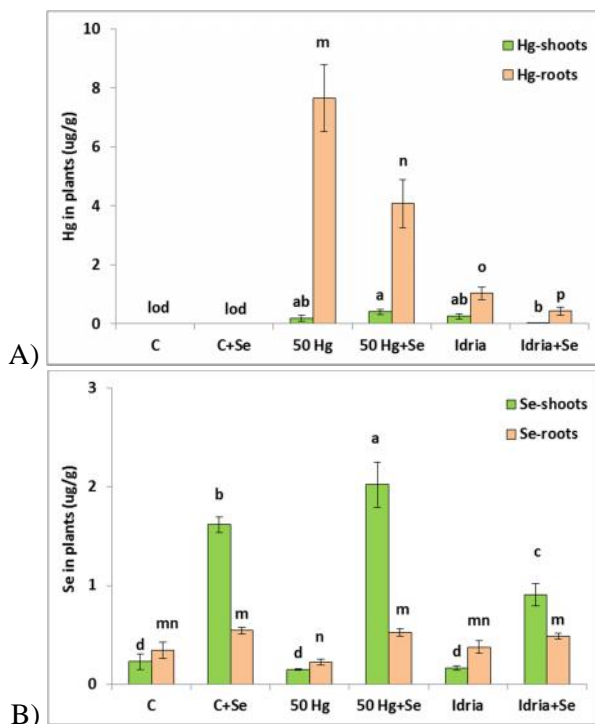


Figure 2. Concentrations of Hg (A) and Se (B) in shoot and root (avr±SE, n=4) as determined by ICP-MS. Different letters above the columns represent

statistically significant differences (a, b, c - shoots; m, n, o - roots), Duncan's post hoc test, $p < 0.05$. lod - the Hg concentrations were below the limit of detection. Se concentration in Se treated plants was significantly higher than in non-treated plants, but still below the toxicity threshold, since no symptoms of toxicity such as chlorosis or necrosis were seen. A trend of Se increase in the shoots was reflected also in the roots (Fig. 2b), indicating that a small proportion of the applied selenate was absorbed in the leaves, reduced in chloroplasts and incorporated into seleno-organic compounds that were transferred to the roots via phloem (White, 2016). Although approximately the same Se contents were applied on the leaves, Se concentrations differed between the Hg treatments (Fig. 2b).

Hg concentration in animals

Animals fed with lettuce that grew in Hg-spiked soil and treated with Se assimilated less Hg compared to solely Hg-treated lettuce (Fig. 3), although Hg concentrations in their food were comparable (3.92 vs. $3.12 \mu\text{g g}^{-1}$). This difference was not observed in animals fed Se-treated lettuce grown in Idrija soil. Interestingly the levels of absorbed Hg were comparable to that of the 50 Hg+Se treatment; although the food concentrations were much lower (0.56 - Idrija vs. 0.23 -Idrija+Se). The differences could be linked to differential Hg speciation and consequently bioavailability in the plants grown in spiked soil vs. plants grown in the soil from Idrija. If there were more reactive/mobile Hg forms in the spiked soil and consequently lettuce, this would lead to higher absorption rates and also Se would be more efficient in binding these species. To confirm this assumption, however, Hg speciation would have to be studied in all the samples. In addition the concentrations of Hg in animals depend also on the animal's biomass (dilution and concentration effects) and feeding rate, which should be taken into account to be able to draw more firm conclusions.

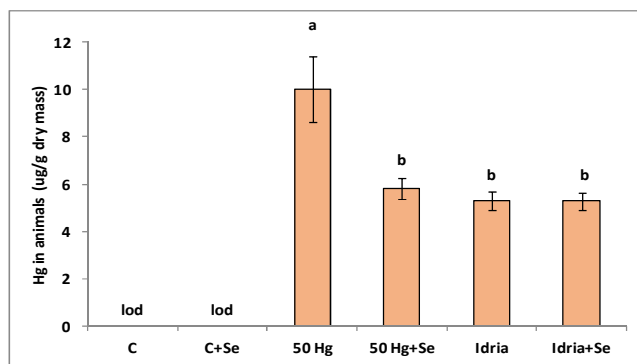


Figure 3: Concentrations of Hg in animals (*Porcellioscaber*) ($\text{avr} \pm \text{SD}$, $n=4$) fed with Hg burdened lettuce, part of which was sprayed with selenium. Different letters above the columns represent statistically significant differences (Duncan's post hoc test, $p < 0.05$). lod - the Hg concentrations were below the limit of detection.

CONCLUSIONS

We have observed that under certain conditions, foliar treatment of plants with selenium could reduce the bioaccumulation of mercury in herbivores. In order to understand the mechanisms that regulate these processes and the potential benefits of using Se to reduce the Hg burden along the food chain, further studies will have to be conducted.

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