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Short communication

The effect of feeding behavior on Hg accumulation in the ecophysiologically different earthworms *Lumbricus terrestris* and *Octolaseon cyaneum*: A microcosm experiment

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Abstract

A soil microcosm experiment was performed to assess the uptake of Hg from various Hg-spiked food sources (soil, leaf litter and root litter of *Trifolium alexandrinum*) by two earthworm species, *Lumbricus terrestris* (anecic) and *Octolaseon cyaneum* (endogeic). Treatments were applied in which one of the three food sources was Hg spiked and the other two were not. Additional treatments in which all or none of the food sources were Hg spiked were used as controls. Uptake of Hg from soil into tissues of both earthworm species was significantly higher than uptake of Hg from leaf litter or root litter, indicating that soil may be the most important pool for the uptake of Hg into earthworms. In addition, the anecic *L. terrestris* significantly accumulated Hg from all Hg-spiked food sources (leaf litter, root litter and soil), whereas the endogeic *O. cyaneum* took up Hg mainly from soil particles. Interestingly, there was no further increase in Hg in *L. terrestris* when all food sources were Hg spiked compared to the single Hg-spiked sources. This may be attributed to the relatively high Hg content in the soil, which may have influenced the feeding behavior of the earthworms, although their biomass did not significantly decline. We suggest that, in addition to the physiological differences, feeding behavior may also play a role in the contrasting uptake of Hg by the two earthworm species.

Keywords: Mercury; Earthworms; Bioaccumulation; Feeding behavior; *Lumbricus terrestris*; *Octolaseon cyaneum*

Mercury (Hg) is a global pollutant (Grigal, 2003) because of its wide distribution by atmospheric transport processes (Jackson, 1997), high toxicity and strong biomagnification within the food chain (Lawrence et al., 1999). With the beginning of industrialisation Hg became enriched in terrestrial ecosystems (Roos-Barraclough and Shotyk, 2003; Schwesig and Matzner, 2000), leading to an accumulation in soils, especially forest soils with their high adsorption capacity (Hakanson et al., 1990; Nater and Grigal, 1992; Godbold, 1994), and in vegetation (Leonard et al., 1998; Grigal, 2003). Despite the increase in Hg in many compartments of the terrestrial ecosystem, only a few studies have examined the transfer of Hg into invertebrates (Beyer et al., 1985; Lawrence et al., 1999).

Earthworms constitute >90% of the invertebrate biomass in soils (Ireland, 1983) and are suggested to be an appropriate tool to predict bioaccumulation in the terrestrial food chain (Rhett et al., 1988; Hinton and Veiga, 2002). Several studies have shown that earthworms accumulate trace metals such as Cu, Pb and Zn (Ma, 1982; Ireland, 1983; Morgan and Morgan, 1988; Bengtsson and Transvik 1989). In contrast, few studies have reported the accumulation of Hg in earthworm tissues (Talmage and Walton, 1993; Edwards et al., 1998). Earthworms ingest dead plant leaves and various soil fractions such as microorganisms, humus and most mineral fractions (Ireland, 1983; Morgan and Morgan, 1992; Cortez and Bouché, 1992), whereas the role of roots as an uptake route of Hg in earthworms remains unclear. Lee (1985) suggested that earthworms may be able to feed on roots and Leonard et al. (1998) stated that roots have higher Hg contents than leaves in general.

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Furthermore, heavy metal accumulation in earthworm tissue was found to depend not only on the concentration of the substrate they consume, but also on ecological and species-specific physiological properties of different earthworms, such as efficiency of detoxification mechanisms, gut morphology, quantity of metal-binding ligands, consumption rates of the food material and feeding behavior (Ma, 1982; Morgan and Morgan, 1992, 1999). However, the relative importance of feeding behavior and physiological properties of earthworms as factors determining heavy metal accumulation in earthworm tissues remains unclear.

The aims of the present study were to determine (1) whether roots, in addition to soil and leaf litter, constitute a relevant uptake route of Hg in the soil-feeding endogeic *Octolaseon cyaneum* and the mostly litter-feeding anecic *Lumbricus terrestris*, and (2) whether physiology or feeding behavior of the two ecologically different earthworm species have strong influence on accumulation of Hg.

A microcosm experiment was established with two different earthworm species and three different Hg-spiked food sources. The experiment comprised five treatments, each having all the three food sources supplied (leaf litter (L), root litter (R) and soil (S)). In three treatments, only one of the food sources was Hg spiked ($L^{Hg}RS$, $LR^{Hg}S$, LRS^{Hg}), one treatment had all sources spiked ($L^{Hg}R^{Hg}S^{Hg}$) and one had none spiked (LRS). The experiment was performed in 3 l-polypropylene containers filled with 1.5 kg of fresh soil. The experimental soil, a sandy loam, was collected from the A-horizon (0–10 cm depth) in a natural beech forest near Schänis (47°9′51″N; 9°4′2″E) in the Swiss Pre-Alps, sieved (2 mm) and thoroughly homogenized. The soil chemical properties were as follows: pH ($CaCl_2$) 5.0, CEC 121 $mmol_c\,kg^{-1}$, N 0.28%, and TOC 2.7%. The soil was spiked (LRS^{Hg}) by homogenously mixing with 300 ml of a 100 μM $HgCl_2$-solution per kg soil or treated with the same amount of distilled water for unspiked soil, resulting in a water content of 27%. Adult individuals of *O. cyaneum* were collected by hand sorting in the same plots from which the soil samples were derived. Individuals of *L. terrestris* were difficult to find in this forest and were therefore obtained from a commercial source. Average background Hg concentrations in the worms reached $0.4\pm0.1\,mg\,kg^{-1}$ dw for *L. terrestris* and $3.9\pm0.6\,mg\,kg^{-1}$ dw for *O. cyaneum*.

To produce Hg-spiked leaves and roots, seeds of *Trifolium alexandrinum* were sown in pots containing the experimental soil and were grown in a greenhouse. This species was chosen because it takes up Hg from soil by accumulation in the roots and leaves and it is a favourite food source for earthworms (Ramadan, 2003). Thirty-day-old plants were treated by watering the plants with a 100 μM $HgCl_2$-solution for 10 d. Finally, plants were harvested and roots and leaves were separated. Both tissues were killed by two freeze–thaw cycles before adding to the experimental containers. The final Hg concentrations of the different food sources are shown in Table 1.

Table 1
Means with standard deviation ($n = 3$) of total Hg concentrations (mg $Hg\,kg^{-1}$ dw) in food sources such as leaf litter, root litter and soil for different treatments at the beginning of the experiment

	LRS	$L^{Hg}RS$	$LR^{Hg}S$	LRS^{Hg}	$L^{Hg}R^{Hg}S^{Hg}$
Leaf litter	0.01±0.01	**6.37±0.09**	0.01±0.01	0.01±0.01	**6.37±0.09**
Root litter	0.15±0.02	0.15±0.02	**44.81±5.27**	0.15±0.02	**44.81±5.27**
Soil	0.11±0.02	0.11±0.02	0.11±0.02	**5.96±0.51**	**5.96±0.51**

Values in bold: total Hg concentrations of Hg-spiked food sources; all others show natural background concentrations.

Each container contained two clitellate individuals of either *L. terrestris* or *O. cyaneum* so that competition for food between the species could be excluded. Worms were fed with Hg-spiked leaf and root litter. In each container, 2.5 g of root litter was placed at a soil depth of 0–10 cm ($LR^{Hg}S$) or 5 g of leaf litter ($L^{Hg}RS$) was distributed on the soil surface. There were three replicates per treatment. Experimental containers were stored in a growth chamber for 35 d in the dark at 20 °C and 60% relative humidity.

At harvest, earthworms were collected and starved on moistened filter papers for 7 d (two specimens in one box; 10 × 10 × 8 cm), so that their gut contents were completely egested. Changes in fresh biomass were determined by weighing earthworms of each treatment at the beginning and at the end of the experiment (weighing of the earthworms occurred after starving on moistened filter papers for 3 d). Earthworms were frozen in liquid nitrogen and lyophilised, ground to a fine powder (3 min at 90% power) using a swing mill (Retsch). The lyophilised earthworms were digested in an UltraCLAVE (MLS Milestone) microwave digestion system in a mixture of HNO_3 (65%) and HF (40%). The soil samples were dried at 15 °C for 7 d and the HNO_3-extractable soil Hg concentrations were determined. Hg in all samples (soil, litter, and earthworms) was determined by cold-vapor atomic absorption spectroscopy using CV-AAS 2100 and FIAS 200-Injection system (Perkin Elmer). Analytical precision was checked against certified standard material and the detection limit of the method was set to 0.01 ng Hg.

Normality of the data was analyzed with the Shapiro–Wilk tests. Normally distributed data were analyzed by analysis of variance (one-way ANOVA) followed by a Tukey post hoc test to analyze for significant differences ($p<0.05$) between treatments using the Origin software version 7 (Origin Lab Corporation).

The concentrations of Hg in earthworms were highest when the soil was Hg spiked compared to the other single-Hg treatments (leaf or root litter) irrespective of the species investigated. In the anecic *L. terrestris*, Hg was taken up from all three Hg-spiked food sources ($p<0.05$) compared to unspiked food (Fig. 1(a)). In contrast, in the endogeic *O. cyaneum* Hg was mainly taken up from soil and not from leaf or root litter ($p<0.05$; Fig. 1(b)). As far as we know this is the first study showing roots as a potential uptake route of Hg in anecic earthworms but not in