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Mercury isotope fractionation for tracing the uptake and metabolism of Hg by earthworms

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Mercury (Hg) is a toxic heavy metal pollutant that can transport globally and accumulate in organism, posing a serious risk to aquatic and terrestrial ecosystems. The exposure and metabolism pathways of Hg in organism are particularly important for understanding the bioavailability and toxicity of Hg. However, previous studies were mostly focusing on the exposure and metabolism of Hg in aquatic biota (e.g., marine mammals and fish), whereas research on terrestrial invertebrates is lacking. Mercury stable isotopes have emerged as a powerful tool for tracing Hg biogeochemical cycling, and has been applied to trace the metabolism and trophic transfer of Hg in aquatic food chains, but the Hg isotope fractionation in terrestrial biota was rarely studied. The goal of this study is to elucidate Hg isotope fractionation during the uptake, metabolism, and elimination of Hg in earthworms, a model terrestrial invertebrate, and provide new insight for the biogeochemical cycling of Hg in terrestrial environment.

Pheretima guillelmi was exposed to Hg-contaminated soils, and Hg concentrations as well as Hg isotope compositions of soils and different tissues of *Pheretima guillelmi* (intestinal contents, epidermis, castings) were analyzed. All tissues displayed more negative $\delta^{202}Hg$ (representing mass-dependent fractionation, MDF) than that in the bulk soils. Among different tissues of *Pheretima guillelmi*, Hg of intestinal contents ($\delta^{202}Hg_{intestinal\text{-}contents}$) were isotopically heavier than epidermis ($\delta^{202}Hg_{epidermis}$) and lighter than castings ($\delta^{202}Hg_{castings}$), suggesting preferential accumulation of lighter Hg isotopes and excretion of heavier isotopes by earthworms. A positive correlation between $\delta^{202}Hg$ and $\Delta^{199}Hg$ in all experimental groups was observed, with a slope about -0.08, suggesting that Hg may have undergone mass independent fractionation (MIF) in internal tissues. We also found that Hg speciation can significantly affect Hg isotope fractionation between different tissues. Overall, our results revealed significant Hg isotope fractionation during the uptake and metabolism of Hg in earthworms, and the fractionation pattern is different from that in aquatic organism, which typically exhibit little MIF and preferential accumulation of heavier Hg isotopes.