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Original article

Short-term pre-exposure increases earthworm tolerance to mercury

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Abstract

To investigate the effects of pre-exposure to mercury, earthworms of the species *Eisenia fetida* Savigny were exposed to either a non-lethal mercury concentration (22 mg/kg soil), test soil, or cultivation medium for one week prior to toxicity testing with mercury. In subsequent tests with exposure to various concentrations of mercury for 4 weeks the effects on survival, reproduction (cocoon production) and glutathione (GSH) levels were recorded. Pre-exposure to mercury strongly increased survival. The 50% lethal concentrations (LC_{50}) for pre-exposed and non pre-exposed earthworms were 545 and 170 mg Hg/kg soil, respectively. Glutathione, which is involved in detoxification of metals such as mercury, was analyzed by a standard spectrophotometric method. Earthworms exposed to mercury concentrations above 22 mg/kg experienced a threefold increase in glutathione levels from a background level of approximately 0.62 μmol/g earthworm wet weight. Glutathione levels were similar in pre-exposed and non pre-exposed earthworms, suggesting that increased glutathione levels did not cause the increased survival of pre-exposed animals. Internal concentrations of mercury were measured with an automated atomic absorbance spectrophotometer, and the uptake rate indicated a one-site binding hyperbola in both pre-exposed and non pre-exposed earthworms. Internal concentrations of mercury reached a maximum of about 150 μg Hg/g earthworm wet weight corresponding to soil concentrations of about 740 mg/kg. The shape of the accumulation curves strongly resembled the ones plotted for internal glutathione levels, suggesting that glutathione levels in earthworms are closely related to internal concentrations of mercury. Critical internal concentrations for effects of mercury on earthworms are also discussed.

Keywords: Glutathione; Mercury; Earthworm; Pre-exposure; LC_{50}; Internal concentration

1. Introduction

Earthworms constitute a major part of the animal biomass in terrestrial ecosystems and have a key role in soil maintenance [2]. Little is known about the toxicity of mercury to terrestrial invertebrates [16] and information on its effects on survival, reproduction, growth or behaviour will be of interest for assessing the risk associated with Hg pollution in terrestrial ecosystems.

Atmospheric deposition is the most significant source of divalent mercury in uncontaminated terrestrial systems, and anthropogenic emissions of Hg have at least doubled global atmospheric Hg deposition rates [9]. Gold and silver mining, as well as the production of products such as

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vermillion and fulminate have historically been major contributors. In more recent times, emissions from coal burning, the chloro-alkali industry as well as mining and smelting of copper and zinc have been the most important anthropogenic sources of mercury. Mercury emissions from anthropogenic sources have plunged since the 1970s, due to environmental and health concerns [10].

The basis for the high toxicity of Hg^{2+} is the affinity it has for sulphydryl groups, which are found in most proteins. Hg^{2+} and -SH form salts which are nearly insoluble and this may cause inhibition of important enzymes, such as membrane ATPases. GSH (a non-protein thiol) is important in the cellular defence against Hg^{2+} and other electrophiles. The -SH group on GSH forms conjugates with Hg^{2+} and is excreted, probably mainly in the form of $Hg(GS)_2$ as this is the most stable for complex of Hg^{2+} and GSH [5,14,27,28,34].

The high affinity of Hg^{2+} for sulphydryl groups suggests that its presence will initially deplete tissues of GSH, but this can be compensated by a subsequent increase in GSH production. Rats exposed to high doses of mercuric chloride have shown significantly reduced levels of GSH in kidneys and kidney mitochondria after relatively short exposure periods [18,20]. An Hg^{2+} challenge will consume GSH in cells, and thereby also reduce the levels of other intracellular antioxidants that compensate for the loss of GSH. Lund et al. [18] propose that Hg^{2+} leads to increased H_2O_2 formation in the mitochondrial respiratory chain. H_2O_2 is metabolised by mitochondrial GSH peroxidase, leading to formation of GSSG in rat kidney mitochondria. Furthermore, Hg^{2+} is known to reduce the activities of superoxide dismutase (SOD) [24] and glutathione peroxidase [25]. Although these effects increase the level of oxidative stress, they may also activate defence mechanisms, such as increased GSH levels via an increased expression of γ-glutamylcysteine synthetase or glutathione reductase [8]. However, this has not yet been studied in earthworms exposed to Hg^{2+}.

The primary objective of this article was to investigate how a short-term pre-exposure to Hg^{2+} affected GSH-levels, biological effects, and internal Hg^{2+} concentrations upon a subsequent exposure of earthworms to Hg^{2+}. Secondly, we wanted to assess the potential of GSH-levels to serve as a biomarker for exposure to Hg^{2+}.

2. Materials and methods

2.1. Test soil

A sandy loam was collected from a research field in Ås, Norway. The soil was dried at 60 °C in a Termaks warming cabinet (Bergen, Norway), and then sieved through a 2 mm mesh to remove stones and plant material. The soil was composed of 76.1% sand (63–2000 mm), 14.6% silt (2–63 mm) and 9.3% clay (2 mm), and the total carbon content was 1.6%. The soil pH and total cationic exchange capacity were 6.2, and 120 mmol(+)/kg, respectively.

2.2. Soil spiking procedure

Mercuric chloride ($HgCl_2$) was purchased from Ferak Labs (Berlin, Germany). Soils were spiked with mercury solutions prepared by diluting a stem solution of 5 mg $HgCl_2$/ml in distilled water to the appropriate concentrations for the experiments: 0 (control), 7.4, 22, 74, 220 and 740 mg/kg soil dry weight. These were thoroughly mixed with soil to ensure an even distribution and the amount of solution was equivalent to 25% of the dry weight of the soil.

2.3. Test organisms

Clitellate specimens of the species *Eisenia fetida*, with an average weight of 0.25 g, were used. Earthworms were cultivated at room temperature in boxes containing a mixture of commercial plant-soil and horse manure.

2.4. Experimental procedure

The three experiments conducted were based on the OECD guideline for earthworm reproduction, with a few adjustments (i.e. non-standard test soil and lower number of earthworms per replicate). In all tests, earthworms were exposed to mercury concentrations of 0, 7.4, 22, 74, 220 and 740 mg/kg for 4 weeks, and four to six replicates were used. Earthworms were depurated on moist filter paper for 24 h before they were allocated randomly in groups of five to the various treatments. The total weight of earthworms added to each replicate was recorded. Test containers were placed in climate rooms at 20 °C with a 16:8 h light/dark cycle. Containers were inspected, fed, and watered once a week. The surviving earthworms and cocoons were counted at test termination, and the earthworms were depurated for 24 h, weighed and snap-frozen liquid nitrogen. Tissue levels of mercury and glutathione were assayed in addition to survival, growth, and reproduction.

As the soil type used in testing was different from the cultivation medium, tests were performed both to determine the effect of introducing a one-week pre-exposure period in test soil and, more importantly, to compare