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Lead accumulation in rats: The effect of the presence of a rat tapeworm and the different forms of metal in the host diet

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ABSTRACT

The main aim of this study was to determine the possibility of using a rat tapeworm, *Hymenolepis diminuta* as a bioindicatortor of organic and inorganic lead forms. The bioaccumulation of cadmium and zinc were determined as well. The influence of this parasite was determined regarding to the concentration of elements in the tissues of a definitive host, the white rat (*Rattus norvegicus*). Male Wistar rats were experimentally infected with *H. diminuta* and exposed to two different forms of lead (lead nitrate and lead bounded in *Pistia stratiotes*) for six weeks via oral exposure of the host. After the exposure period, the element levels were determined in the rat (liver, kidney, spleen, testes, muscles, bones and intestine) and tapeworm tissues with ICP-OES. Tapeworms in Pistia group accumulated 135.2, 98.4, 83.2, 45.1, 38.6 and 25.8 times more Pb concentrations than their hosts muscle, testes, intestine, liver, kidney and spleen, respectively. In Nitrate group, tapeworms accumulated from 2.7 (spleen) to 9.2, 9.5 and 9.6 (testes, liver and muscle, respectively) times higher concentrations than their hosts. Zn was accumulated up to 4.2 times higher in tissues of tapeworm. Cd levels were detected only in tissues of tapeworm, not in their host tissues. Pb concentrations were up to 12.9 times higher in tissues of non-parasitized than in parasitized rats. Lead from lead nitrate accumulated in higher levels than lead from *Pistia stratiotes*. This study confirmed the possibility of using *H. diminuta* as a Pb, Cd and Zn bioindicator of risk element pollution in the environment.

1. Introduction

Metal pollution is currently considered to be one of the most serious global environmental problems. In the body of a living organism, the transport of metals occurs mainly via blood, which distributes them into body tissues like the liver, kidney, brain, lung, spleen, bone or teeth. Metals can accumulated to highly toxic levels and can have a major negative impact on living organisms (Sinicropi et al., 2010).

The overwhelming majority of elements enters into the body of living organisms via oral intake and is absorbed in the digestive tract (Mushak, 1991). This is a place, where many gastrointestinal parasites live, so they come into contact with them. There is high metabolic activity in the whole tapeworm tegument which allows for the intake of nutrients from the host's gut (Dalton et al., 2004).

Through the bioaccumulation of heavy metals in selected organisms, the degree of pollutant toxicity in the corresponding environment can be biomonitored (Zhou et al., 2008). Studies in recent decades indicate that several parasitic helminths are able to bioaccumulate conspicuously higher heavy metal concentrations than the tissues of their hosts (Al-Quraishy et al., 2014; Jankovská et al., 2009, 2016; Nhi et al., 2013; Torres et al., 2004, 2006), which makes them good bioindicators of metal pollution.

Zhou et al. (2008) summarized the characteristics that a perfect metal pollution bioindicator is expected to have, such as accumulation of high levels of pollutants without death, wide distribution, enough abundance, easy sampling or easy raising in the lab. As the rat tapeworm (*Hymenolepis diminuta*) meets most of them, following studies have already been performed to confirm the possibility of using this parasite as a metal pollution bioindicator. The first study on using this tapeworm as a bioindicator was published by Sures et al. (2002), in which they discovered the potential of *H. diminuta* utilization as a lead (Pb) bioindicator. Some field and laboratory studies followed and confirmed the higher Pb uptake by the tissue of parasite than their rat hosts (Sures et al., 2003; Al-Quraishy et al., 2014; Čadková et al., 2014).

A rat tapeworm accumulates Pb in higher concentrations according to Sures (2004), but the bioaccumulation of zinc (Zn) (Jankovská et al.,

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2016), cadmium (Cd) (Teimoori et al., 2014; Jankovská et al., 2016) and chrome (Cr) (Teimoori et al., 2014) has been shown as well. However, the affinity of *H. diminuta* to individual risk elements as well as the bioavailability of these elements in the rat diet should be elucidated to obtain a better understanding of the bioaccumulation ability of this potential bioaccumulator. This can be done only through a laboratory experiment in which tapeworms would be exposed to clearly defined forms and amounts of heavy metals.

Heavy metals can be also absorbed and accumulated in plants (Harguinteguya et al., 2015). Plant sensitivity or tolerance to metal accumulation is influenced by the plant species and genotype. Some plants can phytoaccumulate heavy metals from soil in very high concentrations (Baker and Brooks, 1989). These plants are called hyperaccumulators and can be used for the phytoremediation of soil or water from heavy metals. “Metal hyperaccumulator” is a term that includes plant species that can retain metal concentrations one to three orders of magnitude greater than non-hyperaccumulators (Ashraf et al., 2011). *Pistia stratiotes* (known as water lettuce, water cabbage, shellflower or Nile cabbage) is a free-floating pleustonic macrophyte that can hyperaccumulate several heavy metals, especially Pb (Veselý et al., 2012).

The aim of this study was i) to verify the possible use of *H. diminuta* as a bioindicator of lead intake by the host organism as affected by lead bioavailability in the diet and ii) to assess the influence of parasitism on the accumulation of risk elements in the tissues of a definitive host, the white rat (*Rattus norvegicus*).

2. Material and methods

2.1. Experimental infection of rats

This study was carried out using 36 male Wistar rats, weighing approximately 200 g each, obtained from a commercial source (Velaz, Czech Republic). Mealworm beetles (*Tenebrio molitor*) from the Department of Zoology and Fisheries experimental breeding program were used for infection with *H. diminuta*. Several tapeworm eggs were given to the beetles perorally and a two week development of infective cysticercoids followed at 29° C. Full-grown cysticercoids were collected from beetles and were subsequently perorally administered to rats. Each rat was given 3–5 cysticerkoids. Rats were housed in standard E4 boxes with a constant temperature (22 ± 2 °C) and humidity (50 ± 2%), fed with commercial pellet complete food for mice and rats in barrier cages (ST-1, Velaz, Czech Republic) and given water *ad libitum* for 5 weeks. After 5 weeks, a coproscopic examination confirmed the presence of mature parasites in the rat intestines.

2.2. Experimental design

After the presence of *H. diminuta* in rats was confirmed, rats were housed individually in metabolic cages for six weeks, kept on a 12 h light/dark cycle with a constant temperature (22 ± 2 °C) and humidity (50 ± 2%), fed ST-1 commercial pellet food and given water *ad libitum*. Experimental rats were subdivided into three groups (Table 1). Rats in the control (C + CT) group (n = 12) were fed only pellet food and were not exposed to an increased amount of any heavy metal. Rats in the nitrate (N + NT) group (n = 12) were fed pellet food and were administered 6 mg of Pb in the form of lead nitrate ($Pb(NO_3)_2$) solution by oral gavage once per a week. Rats in the *Pistia* (P + PT) group (n = 12) were fed pellet food and were administered *P. stratiotes* mixed with water by oral gavage once a week. Six rats in each group were parasitized by *H. diminuta* and six rats were not infected with parasites. The amount of *P. stratiotes* in solution given to rats was calculated in such way that each rat was given 6 mg of lead weekly. Some other elements, e.g. zinc (Zn) or in small amount cadmium (Cd) are naturally bound to this plant (Table 2); therefore, these rats were also exposed to other elements in higher concentrations than the control or nitrate groups. Total element intake during the exposure period is shown in Table 1.

Table 2
Concentration of elements in *Pistia stratiotes* (mg/kg of dry weight) given to rats.

Element	Pb	Zn	Cd	Fe	Mn	Cu
Concentration	68967	145	1,13	1836	435	39

2.3. Preparation of lead (Pb)

Pb nitrate for the nitrate group was obtained from a commercial source (Sigma Aldrich, Lachema, Czech Republic.). Individual plants of *P. stratiotes* were cultivated in a water solution enriched with 3200 ± 40 mg of Pb per liter. Plants were cultivated in plastic barrels in the experimental greenhouse of Czech University of Life Sciences in Prague. The Pb concentration in the cultivation medium was regularly measured by an inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA). After harvesting, plants were carefully washed with double-distilled water, weighed, frozen and freeze-dried. The plant material was then homogenized into a powder and the total concentration of Pb and other elements was determined using ICP-OES.

2.4. Sampling and element determination

All rats were euthanized after six weeks of exposure with a combination of anesthetic and analgesic. Samples of tissue were taken from the liver, kidney, spleen, testes, muscles, bones and small intestine wall with a Teflon® tools. Also, tapeworms were taken from the small intestine of rats. Collected tissues were carefully washed in double-distilled water, weighed, frozen and subsequently freeze-dried and homogenized. Tissue samples were mineralized by wet digestion through the use of microwave heating under increased pressure (microwave oven Ethos 1, Milestone, Germany) for 25 min at 231 °C in 6 ml of 65% p.a.$^+$ nitric acid and 2 ml of p.a. hydrogen peroxide (Analytika Ltd., Prague, Czech Republic). After digestion, the excess acid was blown off into an extractor fan. Subsequently, samples were transferred into a 50 ml volumetric flasks and filled up with deionized water. Blanks, prepared using the identical procedure as in the case of

Table 1
The total intake of elements (mg) by rats in individual groups.

Group	Number of rats	Total intake of (Pb mg)	Total intake of Cd (mg)	Total intake of Zn (mg)	Total intake of Fe (mg)	Total intake of Cu (mg)	*H. diminuta*
Control (C)	6	1.64	0.12	69.45	301.07	20.14	−
Control + tapeworm (CT)	6	1.64	0.12	69.45	301.07	20.14	+
Nitrate (N)	6	37.6	0.12	69.45	301.07	20.14	−
Nitrate + tapeworm (NT)	6	37.6	0.12	69.45	301.07	20.14	+
Pistia (P)	6	37.6	0.20	69.53	302.03	20.16	−
Pistia + tapeworm (PT)	6	37.6	0.20	69.53	302.03	20.16	+

C, CT − control group, N, NT − nitrate group, P, PT − *Pistia* group.