

How the tapeworm *Hymenolepis diminuta* affects zinc and cadmium accumulation in a host fed a hyperaccumulating plant (*Arabidopsis halleri*)

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Abstract The effects of plant-bound zinc (Zn) and cadmium (Cd) on element uptake and their interactions in a parasite-host system were investigated in a model experiment. Male Wistar rats were divided into four groups (C, P, TC and TP). Groups TC and TP were infected with the rat tapeworm *Hymenolepis diminuta*. Groups C and TC were fed a standard rodent mixture (ST-1) and received 10.5 mg of Zn per week, while groups P and TP were fed a mixture supplemented with the Zn- and Cd-hyperaccumulating plant *Arabidopsis halleri* at a dosage of 236 mg Zn/week and 3.0 mg Cd/week. Rats were euthanized after 6 weeks, and Cd and Zn levels were determined in rat and tapeworm tissue. The results indicate that tapeworm presence did have an effect on Cd and Zn concentrations in the host tissue; the majority of tissues in infected rats had statistically significant lower Zn and Cd concentrations than did uninfected rats. Tapeworms accumulated more zinc and cadmium than did the majority of host tissues. This important finding confirms the ability of tapeworms to accumulate certain elements (heavy metals) from the host body to their own body tissues. Thus, tapeworms can decrease heavy metal concentrations in host tissues.

Keywords Rat · Tapeworm · Plant · Accumulate · Cadmium · Zinc

Introduction

Risk element contamination of the environment is a global problem (Brozova et al. 2015; Jankovska et al. 2014; Oprsal et al. 2015; Vaculik et al. 2015; Zarubova et al. 2015).

However, zinc, copper, chromium, manganese and cobalt are also essential trace elements. These are defined as elements contained in low concentrations (mg/kg or less) in plants and animals (Phipps 1981). They are essential for metabolic processes in animals, but at higher levels, they may be toxic. Zinc ensures proper development and an effective immune response. Zinc is the central atom of a wide range of metalloenzymes, and as a part of the insulin molecule, it interferes with the metabolism of sugars (Brown et al., 2001; Brody, 1998; Cuajungco and Lees, 1997; Frederickson et al., 2005; Sun et al. 2011). When zinc is taken at high doses for the long-term, it is absorbed at the expense of other metals, and symptoms of anaemia may develop (WHO 1996; FAO 2001; Hotz and Brown 2004).

Cadmium is a toxic element that is primarily acquired through food consumption. Intestinal absorption of Cd is proportional to its concentration in the diet; however, other factors also influence the rate of the intestinal absorption and organ retention of Cd. One significant problem is the interaction between Cd and other mineral nutrients that are antagonistic to Cd absorption (Reeves and Chaney 2008).

Biogenic (Cu, Cr, Co, Mn, Zn) and toxic (Pb, Cd, Hg) elements in soil can be released (under suitable physico-chemical conditions) and become potentially available for plants in areas with elevated levels of these elements. Thus, soil-plant transfer is a possible way for these metals

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to become part of the food chain (Cadkova et al. 2013). Certain plants, including *Arabidopsis halleri*, can tolerate or even accumulate very high concentrations of cadmium and zinc. Such plants are known as hyperaccumulators and can considerably accelerate the introduction of soil-bound toxic elements into the food chain. Moreover, these species can also be used in phytoremediation and the monitoring of heavy metals in the environment (Zhenli et al., 2005). The majority of elements found in animals enter through the oral route and are subsequently absorbed in the digestive tract. This absorption process significantly interferes with gastrointestinal parasites, especially acanthocephalans (and tapeworms), which receive nutrients through the tegument, a metabolically active body surface (Sures et al., 2000a, Sures et al., 2000b, Sures et al., 2002; Eira et al. 2005; Kosik-Bogacka et al., 2010). Tapeworms are able to accumulate a considerable amount of metals and reduce their concentrations in host tissues (Jankovska et al. 2011; Cadkova et al. 2013).

The aim of the present study was to investigate the ability of the rat tapeworm *Hymenolepis diminuta* to not only accumulate cadmium and zinc derived from the hyperaccumulating plant *A. halleri* but also affect their concentrations in the tissues of a definitive host (*Rattus norvegicus*).

Material and methods

Breeding and infection of rats

Twenty-four male Wistar rats (*R. norvegicus* var. *alba*) each weighing 150 g, were immediately checked for the presence of the intestinal helminths through a faecal sampling examination.

Rats were divided into four groups of six individuals and kept at a temperature of 21 ± 2 °C and a relative humidity of 75 ± 5 % for 3 weeks. This 3-week period is required for rats to acclimatize and for tapeworms to fully develop in the infected rats. During the acclimatization period (3 weeks), rats were given ad libitum access to both water and a standard ST-1 rodent feed, commercially available from Velaz Ltd. (Table 1).

Infection of rats was initiated with cysticercoids acquired from laboratory-bred beetles (*Tribolium confusum*), which were infected by ingesting tapeworm eggs collected from the excrements of previously infected rats. Cysticercoid development in beetles took place over a 20-day period. The cysticercoids were then collected, suspended in a solution of glucose and administered to the rats orally via micropipettes. Each rat was infected with three cysticercoids.

Table 1 Composition of ST-1

Moisture	%	12.5
Nitrogen compounds	%	24
Fibre	%	4.4
Lipids	%	3.4
Ash	%	6.8
Lysin	mg/kg	14,000
Methionin	mg/kg	4800
Ca	mg/kg	11,000
P	mg/kg	7200
Na	mg/kg	1800
Cu	mg/kg	20
Zn	mg/kg	70
Se	mg/kg	0.38

Experimental design

When tapeworm infection was verified, the rats were housed individually in metabolic cages with a controlled temperature of 21 ± 2 °C and relative humidity 75 ± 5 %. Mode light was set at a 12 h/12 h dark/light cycle. Rat group distribution is shown in Table 2. Over a period of 6 weeks, groups P and TP were given ST-1 (25 g/day) supplemented with dried and homogenized *A. halleri* containing 50.4 mg/kg of Cd and 3912 mg/kg of Zn. Therefore, both groups P and TP received weekly zinc and cadmium doses of 236 and 3.0 mg, respectively. Groups C and TC received only finely minced ST-1, which provided only 10.5 mg of zinc per week (we fed rats only 6 days a week; the seventh day was a fasting day, when rats were provided with water only). Animal body weight was monitored weekly. The EU Legislation limits Zn content in complete feed mixtures to 250 mg/kg (EU regulation 2316/98), i.e., 37.5 mg/kg/week.

Sampling and analytical determination of metals

Six weeks into the study, the rats were euthanized and tissues were taken from the following seven organs with Teflon tools: the liver, small intestine, kidneys, spleen, muscle, testes and

Table 2 Experiment design

Group	n of animals	Zn/week (mg)	Cd/week (mg)	<i>H. diminuta</i>
Control (C)	6	10.5	–	–
Control + tapeworm (TC)	6	10.5	–	+
Exposure (P)	6	236	3.0	–
Exposure + tapeworm (TP)	6	236	3.0	+

bone tissue (marrow and osseous tissues). Furthermore, tapeworms were removed from the small intestines of the infected rats. All samples were immediately placed in a freezer at -20°C and subsequently freeze-dried. The samples were then pulverized, and aliquots taking approximately from 400 to 500 mg were decomposed through microwave-assisted digestion using a mixture of 65 % HNO_3 (8.0 ml) and 30 % H_2O_2 (2.0 ml) purchased from Analytica Ltd., Prague, Czech Republic by using the device Ethos 1 (MLS GmbH, Leutkirch, Germany) at 220°C for 45 min. The digests were poured into 20-ml glass tubes and diluted to 20 ml with distilled water. Certified reference material BCR 185R bovine liver was added to the samples for quality assurance analysis.

Element contents in the digests were determined by inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber and a V-groove pneumatic nebulizer made of inert material. To determine low Cd concentrations in the digests, we implemented electrothermal atomic absorption spectrometry (ETAAS) through the use of a VARIAN AA280Z (Varian, Australia) equipped with a GTA120 graphite tube atomizer.

Statistical analysis

Element concentrations and their statistical differences were compared between groups using the nonparametric Mann-Whitney U test. All computations were done using Statistica 10 software (Statsoft, USA).

Results and discussion

Our results indicated statistically significant differences between groups of rats infected with tapeworms and their non-

infected counterparts (Figs. 1, 2, 3 and 4). The rat group affected by both the Cd- and Zn-hyperaccumulating plant and tapeworms (TP) had significantly lower ($p < 0.01$) concentrations of cadmium and zinc in the majority of their tissues when compared to the non-infected rat group (Figs. 2 and 4).

It was determined in the 1990s that several helminths (primarily acanthocephalants in fish) are able to accumulate considerable concentrations of heavy metals (Lafferty 1997; Sures et al. 1998; Sures 2001; Sures and Siddall 2001; Sures 2003; Thielen et al. 2004). Information regarding whether parasites in terrestrial vertebrates can serve as sentinels for heavy metal environmental pollution, as well as the benefits they provide to their hosts, remains inconsistent (Sures et al. 2002; Baruš et al. 2003; Torres et al. 2004, 2006; Jankovská et al., 2008, Jankovska et al., 2009, Jankovská et al., 2010). Since cestodes are more abundant in terrestrial mammals than are acanthocephalans and, thus, potentially more useful in passive as well as active biomonitoring; a very common animal (*R. norvegicus*) and its common tapeworm (*H. diminuta*) were selected for the present study. As Sures et al. (2002) reported in their lead biomonitoring study, this host-parasite model can be used both as a bioindicator to monitor environmental pollution (especially in urban areas) and as a means to reduce heavy metals in the organs and tissues. As Sures et al. (2002) did with their study dealing with lead concentrations, we compared Cd and Zn concentrations accumulated by the host and those in tapeworm tissues (bioconcentration factor $\text{BF} = C(\text{tapeworm})/C(\text{host tissue})$).

With respect to Zn concentrations, tapeworms accumulated 160.34 (TC) and 200.26 (TP) mg kg^{-1} . This translates to 1.9, 2.1, 2.0, 1.1, 2.2 and 4.0 times more Zn than that accumulated in the liver, spleen, kidneys, bone, small intestine and muscles, respectively, of the host from the TC group. Only testis tissue accumulated slightly more zinc than the tapeworms did (Table 3). This can be

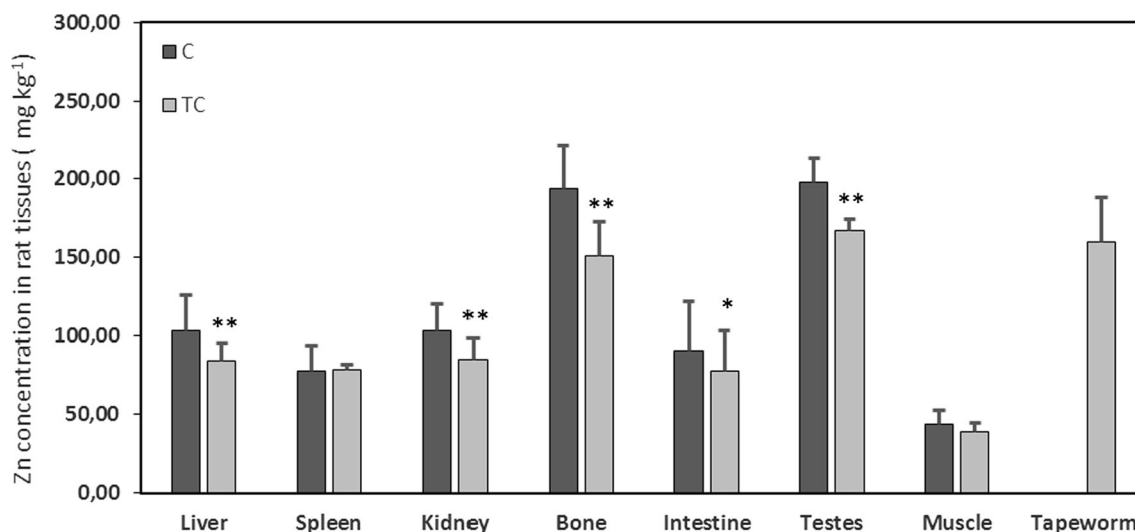


Fig. 1 Zinc concentrations in the tissues of rats fed a standard mixture ST-1 (C) and of rats infected by tapeworms (TC) * $p \leq 0.05$ ** $p \leq 0.01$

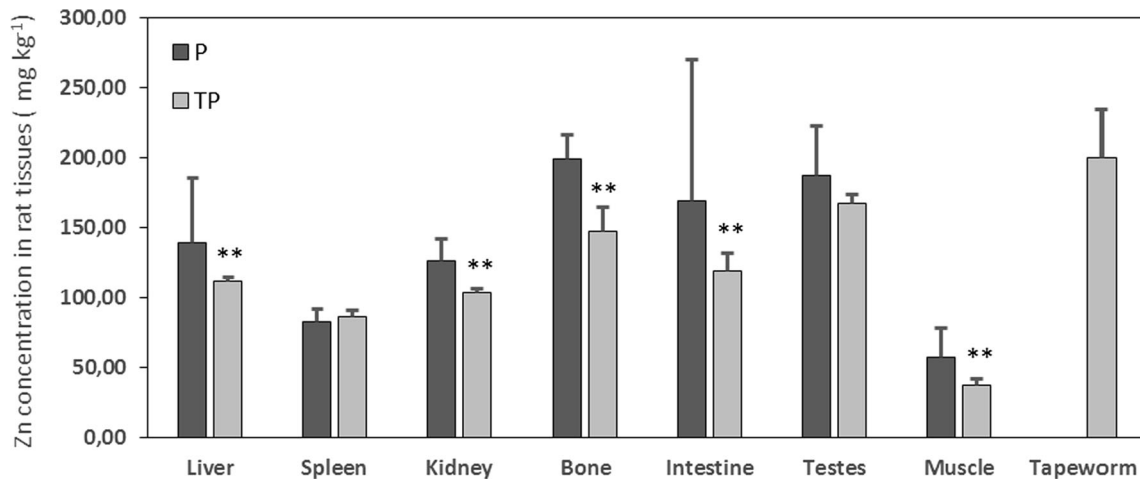


Fig. 2 Zinc concentrations in the tissues of rats fed *Arabidopsis halleri* (P) and of rats infected by tapeworms (TP) $**p \leq 0.01$

attributed to the protective effects of Zn against testicular damage caused by Cd (Bonda et al., 2004).

It is known that feeding high concentrations of zinc, iron and/or calcium to animals reduces the rate of absorption of cadmium from various food sources. When zinc is marginal in the diet, the delay of cadmium excretion is more pronounced (Reeves and Chaney, 2004). The rates of absorption and whole-body retention of dietary Cd increased 7- to 10-fold when experimental animals were fed diets containing marginal concentrations of Zn, Fe and/or Ca (Reeves and Chaney, 2001, 2002).

In *A. halleri* leaves, Zn is bound mainly to malate or other organic acids (Sarret et al. 2009); Cd is also bound to organic acids, cell wall components and, to a lesser extent, thiol-containing molecules (Huguet et al. 2012). Previously published papers have indicated that metals in plants are more easily absorbed than those in inorganic forms, which are artificially added to animal feed (Cadkova et al. 2013). To our knowledge, Válek et al. (2015) were the first to use *A. halleri* in

a feeding study. Recent studies have suggested that using *A. halleri* in feed stresses the consumer organism due to its Cd content, rather than its Zn content. Cadmium (Cd) is an environmental pollutant that is ranked eighth among the top 20 most hazardous substances (Klaassen et al. 2009), and human activity has markedly increased its distribution in the global environment. Zinc is an essential element for all organisms. However, it is toxic when taken in excess (Johnson et al. 2007).

In Tables 3 and 4, we compared zinc and cadmium concentrations between organs of rats with or without parasites and with or without *A. halleri* diet supplementation. Cd concentrations were significantly higher in rats given *Arabidopsis* in their feed mixture (group P); this group (P) had Cd levels that were 329, 147, 87, 39, 10 and 3 times higher in the kidneys, liver, small intestine, testes, spleen and muscle, respectively, than in those of rats not given *Arabidopsis* (group C). Cadmium concentration differences between groups C and TC, as well as between P and TP, are presented in Table 4. There were only slight zinc concentration differences between

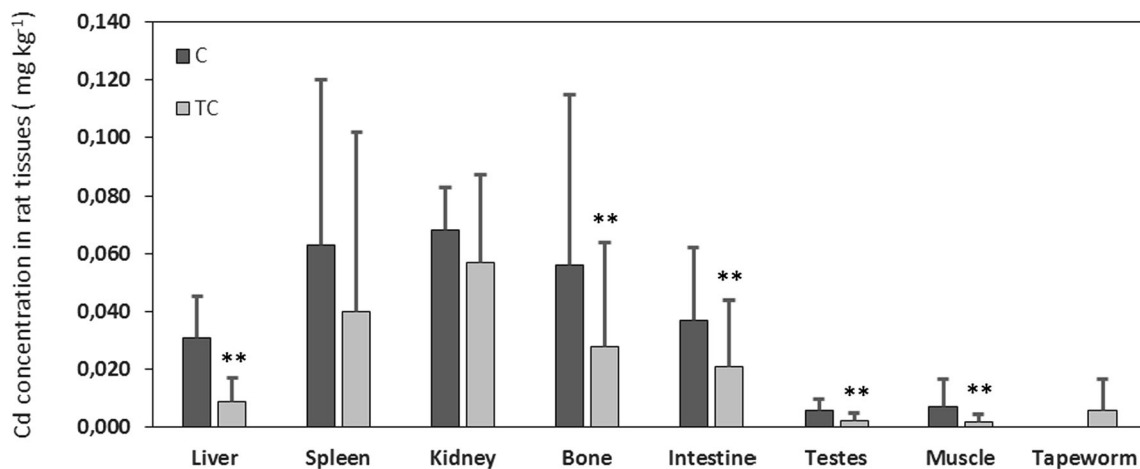


Fig. 3 Cadmium concentrations in the tissues of rats fed a standard mixture ST-1 (C) and of rats infected by tapeworms (TC) $**p \leq 0.01$

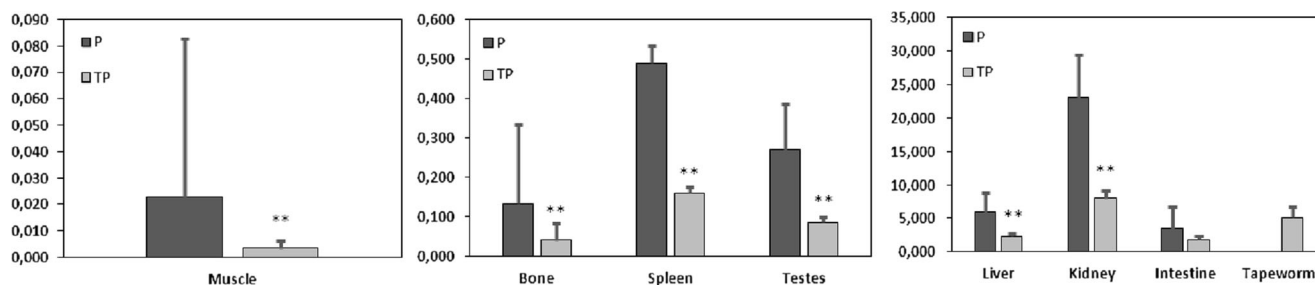


Fig. 4 Cadmium concentrations in the tissues of rats fed *Arabidopsis halleri* (P) and of rats infected by tapeworms (TP) $**p \leq 0.01$

groups C and TC, P and TP, as well as between P and C, ranging from ratios of 0.9 (testes) to 1.7 (small intestine tissue) as presented in Table 3.

The main site of zinc absorption in animals is the small intestine, where the distal duodenum and proximal jejunum play a key role. Zinc excretion is primarily through faeces (1–5 mg of Zn can be excreted by humans over a 24-h period). Zn levels are directly influenced by the content of zinc in the diet. Stools contain unabsorbed zinc from food, endogenous zinc secreted into the intestine from the pancreas and gallbladder and zinc from the intestinal epithelial cells (Krebs, 2000).

The liver, kidneys, bones and testes of group TC had significantly lower Zn concentrations ($p < 0.01$) than did those of group C (Fig. 1); statistically significant differences between these two groups were also found in the small intestine ($p < 0.05$).

The liver, bones, small intestine, testes and muscles of rats with tapeworms (TC) had significantly lower Cd concentrations (Fig. 3) than did those of group C (non-infected rats).

The liver, kidneys, muscles, bones, testes and spleen of rats infected with tapeworms and fed *A. halleri* (TP) had significantly ($p < 0.01$) lower Cd concentrations (Fig. 4) than did those of the non-infected rats (P). In the TP group, tapeworms accumulated Zn concentrations that were 1.8, 2.3, 1.9, 1.4, 1.7, 1.2 and 5.5 times higher than those accumulated by the liver, spleen, kidneys, bones, small intestine, testes and muscles, respectively (Table 3). Zn concentrations were lower in all host tissues

than in the tapeworms. This supports the theory regarding the ability of tapeworms to accumulate heavy metals from the host.

Scheef et al. (2000) described the ability of the acanthocephalan parasite (*Moniliformis moniliformis*) to accumulate cadmium from its rat host (*R. norvegicus*). The study lasted 3 weeks, and the rats were exposed to a solution of CdCl₂. They found that the parasite accumulated significantly more of this element than did rat tissues. However, there was no indication that cadmium levels in the tissues of infected rats were significantly lower than those in non-infected rats. Similar results were published by Sures et al. (2000b) in the case of lead, another risk element. They investigated the acanthocephalan parasite *M. moniliformis*, which parasitizes in rats, and found that it accumulated Pb from the host body. They determined that acanthocephalan females contained 25, 39, 2 and 9 times more Pb than did the host liver, small intestine, kidney cortex and kidney medulla, respectively. The ratio of acanthocephalan males was different (7; 11; 0.5 and 3). However, tapeworms are hermaphrodites, so our study could not provide such comparisons. It is evident from both experiments that acanthocephalans have the ability to accumulate higher concentrations of metals than do the host tissues.

In the case of cadmium, tapeworms accumulated 2.2 and 2.6 times higher levels than did the host testis and muscle tissue, respectively (group TC). The remaining host tissues contained higher Cd concentrations than did

Table 3 Zinc concentrations in rat tissues (mg kg⁻¹) and bioconcentration factors (BF)

	Liver	Spleen	Kidney	Bone	Intestine	Testes	Muscle	Tapeworm
C	110.65	77.01	111.08	190.22	102.20	205.33	44.89	
TC	86.08	76.97	82.38	150.86	72.83	167.32	39.79	160.34
BF	1.9	2.1	2.0	1.1	2.2	0.96	4.0	
C/TC	1.3	1	1.4	1.3	1.4	1.2	1.1	
P	139.34	82.92	126.52	199.45	168.96	186.82	57.24	
TP	111.65	85.82	103.16	146.75	119.26	167.55	36.68	200.26
BF	1.8	2.3	1.9	1.4	1.7	1.2	5.5	
P/TP	1.3	1	1.2	1.4	1.4	1.1	1.6	
P/C	1.3	1.1	1.1	1.1	1.7	0.9	1.3	

BF (bioconcentration factor = concentration in tapeworm/concentration in host tissue) is the concentration accumulated by the host and those in tapeworm tissues; C/TC, P/TP, P/C is the share (ratio) of individual groups (C, P, TC, TP)

Table 4 Cadmium concentrations in rat tissues (mg·kg⁻¹) and bioconcentration factors (BF)

	Liver	Spleen	Kidney	Bone	Intestine	Testes	Muscle	Tapeworm
C	0.04	0.047	0.07	0.10	0.04	0.007	0.009	
TC	0.01	0.05	0.05	0.02	0.01	0.003	0.003	0.006
BF	0.6	0.1	0.1	0.3	0.5	2.2	2.6	
C/TC	4	0.9	1.4	5	4	2.3	3	
P	5.87	0.49	23.01	0.13	3.46	0.27	0.023	
TP	2.36	0.16	7.94	0.04	1.86	0.09	0.003	5.09
BF	2.2	32.0	0.6	127.6	2.7	59.6	1551.6	
P/TP	2.5	3.1	2.9	3.3	1.9	3	7.7	
P/C	147	10	329	1.3	87	39	3	

BF (bioconcentration factor = concentration in tapeworm/concentration in host tissue) is the concentration accumulated by the host and those in tapeworm tissues; C/TC, P/TP, P/C is the share (ratio) of individual groups (C, P, TC, TP)

the tapeworms (Table 4); however, there were only trace quantities in this case since rats from the TC group were not affected by Cd in food.

Group TP rats were affected with cadmium through diet, and the tapeworm Cd concentrations for this group were 2.2, 32.0, 127.6, 2.7, 59.6 and 1551.6 times higher than those in the liver, spleen, bone, small intestine, testes and muscles of host, respectively (Table 4). The kidneys are major Cd-accumulating organs in mammals. This was confirmed by our study; Cd concentrations in kidneys reached 7.94 mg kg⁻¹, which was 1.6 times higher than those in tapeworms (Table 4).

There is not sufficient scientific literature concerning the behaviour of the rat tapeworm *H. diminuta* in the presence of cadmium or zinc in a host. Sures et al. (2002) studied the effects of rat tapeworms (*H. diminuta*) on laboratory rats exposed to lead as Pb(CH₃COO)₂. After calculating the bioconcentration factor, they found lead concentrations in the tapeworms that were 17 times higher than those found in the rat kidneys.

Our study determined Zn concentrations in tapeworms that were 1.9 times higher than those in the host kidneys (Table 3, group TP). Contrarily, Cd concentrations in the kidneys of hosts from the same group (TP) were 2.85 mg kg⁻¹ higher than those in the tapeworms (Table 4).

Nevertheless, our results showed that tapeworms have a significant effect on zinc and cadmium accumulation in host (rat) tissues. Even though Zn concentrations were similar in both groups (with or without *Arabidopsis*), Cd concentrations were significantly higher in rats given *Arabidopsis* in their feed mixture (group P); this group (P) exhibited Cd levels that were 329, 147, 87, 39, 10 and 3 times higher in the kidneys, liver, small intestine, testes, spleen and muscle, respectively, than in those of rats not given *Arabidopsis* in their feed mixture (group C). Tapeworms accumulated more zinc and cadmium than did the majority of host tissues. For example, tapeworms accumulated 5.5 times more Zn and 1542 times more Cd than did the host muscle tissue. Moreover, when we compared group TC (standard feed mixture and tapeworm

infection) with group TP (feed mixture with added hyperaccumulating plants and tapeworm infection), we found that tapeworms from group TP accumulated 848 times more Cd than did tapeworms from group TC.

Since few comparative studies on heavy metal concentrations in tissues of infected and uninfected hosts are available, it remains unclear if conspicuous metal accumulation by parasitic worms affects metal levels in the tissues of the definitive host.

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Compliance with ethical standards All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on the Protection of Animals against Cruelty and EC Directive 86/609/EEC.

Conflict of interest The authors declare that they have no conflict of interest.

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