Is the tapeworm able to affect tissue Pb-concentrations in white rat?

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SUMMARY

The effect of gastrointestinal helminths on Pb accumulation in the host body is ambiguous. A laboratory experiment with *Rattus norvegicus/Hymenolepis diminuta* model was conducted to determine Pb toxicokinetics in a terrestrial host-parasite system. The ET-AAS or ICP–OES techniques were used to determine Pb concentrations (C_{Pb}) in both tapeworms and host tissues (kidney, liver, bone, testes, muscle and intestinal wall). Concerning the entire host-parasite system, the highest C_{Pb} were detected in *H. diminuta*. Rat kidneys and bone were the only two tissues whose mean Pb levels were lower in parasitized animals than they were in non-infected subjects after both levels of exposure. At low Pb exposure, parasitization slightly changed the Pb toxicokinetics in the host body. However, with respect to tissue at the same exposure level, no significant differences were detected between the parasitized and non-parasitized animals and no significant correlations were found between C_{Pb} in tapeworms and those of host tissues. The results of this study indicate that *H. diminuta* does not protect rat from elevated Pb exposure even if tapeworm accumulates a higher portion of ingested Pb dose compared with that of the most Pb-loaded host soft tissue. The portion of Pb dose accumulated in *H. diminuta* correlates positively with parasite biomass.

Key words: *Hymenolepis diminuta*, tissue Pb levels, Pb oral exposure, bioaccumulation, Pb toxicokinetics, heavy metals, bioconcentration factor.

INTRODUCTION

Recent interdisciplinary parasitological/toxicological research has confirmed that gastrointestinal helminths (GIH) absorb certain heavy metals and risk elements from host intestinal content (de Buron *et al*. 2009). Primarily, adult stages of Cestodes and Acanthocephalans accumulate these elements to a greater extent than do their host tissues (Schludermann *et al*. 2003; Torres *et al*. 2004, 2006; Retief *et al*. 2006; Jirsa *et al*. 2008). Even though environmental pollution may adversely affect the GIH assemblages (Oros and Hanzelova, 2009), simultaneous treatment of helminths and contaminant exposure in wild animals is common in natural habitats (MacKenzie *et al*. 1995; Quadroni *et al*. 2013). Therefore, the use of GIH as accumulative indicators of environmental contamination is frequently discussed in scientific literature (Sures, 2004; Vidal-Martinez *et al*. 2010; Nhi *et al*. 2013).

Beyond this, numerous recent studies have observed effects of GIH presence on contaminant (especially heavy metals) concentrations in animals, which are used in routine biomonitoring of aquatic (Sures *et al*. 1997, 1999*a*, *b*; Sures and Siddall, 2001; Azmat *et al*. 2008) or terrestrial (Sures *et al*. 2003*b*; Torres *et al*. 2004, 2006, 2010; Jankovská *et al*. 2009) environments. The majority of these studies deduced from hypothesis that if an organism is exposed to a specified amount of contaminant, and a certain portion of this dose is accumulated by GIH, only an impaired level of pollutant may be available for absorption and accumulation in host tissues. Under these circumstances, lower concentrations of contaminants may be detected in host tissues if some GIHs are present in their alimentary tract. For this reason, data describing environmental burden may be distorted, as indicated by Sures *et al*. (2003*a*) and Jankovská *et al*. (2009).

However, previous studies have provided contradictory results concerning the effect of GIH parasitization on final heavy metals concentration in host tissues. In certain cases, the C_{Pb} in the tissues of parasitized animals exceeded those of non-parasitized subjects (Jankovska *et al*. 2010*a*, *b*; Khaleghzadeh-Ahangar *et al*. 2011). For a better understanding of GIH and heavy metal exposure interaction, we

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Table 1. Experiment design

Group	N of hosts	*Hymenolepis diminuta*	Pb treatment	Total Pb intake (average ± S.D. in the group)
OO	6	–	–	1 200[a] μg of Pb
TO	6	+	–	1 200[a] μg of Pb
OMt	6	–	+	32 100 ± 713 μg of Pb
TMt	6	+	+	32 500 ± 851 μg of Pb

[a] computed: average feed intake (15 g) per day × pellet food Pb content (2 mg kg^{-1}) × duration of experiment (40 days).
T, tapeworm *H. diminuta*; Mt, metal (lead acetate – trihydrate); O, no tapeworm/no exposure.

conducted a laboratory experiment under controlled conditions (specified Pb doses of clearly defined Pb compound, host of uniform age, sex and weight, uniform access to feed and standardized climatic conditions). A common host-parasite system, *Rattus norvegicus*/*Hymenolepis diminuta*, was used in this study in order to determine the effect of tapeworm parasitization on CPb in terrestrial host tissues.

MATERIALS AND METHODS

Experimental animals

Twenty-four male Wistar rats at 2 months of age (weighting 250–30 g) were obtained from a commercial supplier (Physiological Institute, Academy of Sciences, Prague, Czech Republic). Animals were housed individually in conventional cages (2154F, Trigon-plus, CR), kept under standardized conditions (22 ± 2 °C, 12/12 h dark/light cycle), fed commercial pellets (An-Lab, Prague, Czech Republic) and allowed to drink tap water *ad libitum*. Maintenance of all experimental animals was carried out in compliance with the current laws of the Czech Republic (Act No. 246/1992 coll. on Protection of animals against cruelty) and the European Union (EC Directive 86/609/EEC). The minimum number of experimental animals was used to achieve appropriate results. Animals were acclimatized for 7 days prior to commencement of the experiment and subsequently divided into four groups depending on parasitization/exposure combination. Detailed information regarding experiment design is listed in Table 1.

Experimental inoculation

Hymenolepis diminuta eggs were separated from feces of *H. diminuta* positive adult rats (experimental strain of the Department of Zoology and Fisheries, Czech University of Life Science, Prague). Adult *Tenebrio molitor* were used as intermediate hosts. Thirty flour beetles were starved for 24 h and subsequently placed into Petri dishes, where a glucose solution containing approximately 10 000 tapeworm eggs was applied on filter paper. Beetles were left for 24 h in this condition. Afterward they were fed oat flakes *ad libitum* and kept at 28 °C in a plastic box. Twelve days later, successful inoculation was confirmed through an autopsy of 3 beetles, in which fully developed cysticercoids were found. The inoculation of 12 rats was carried out by feeding each rat with 2 inoculated beetles. Tapeworms were left for 5 weeks to develop to the adult stage. The presence of *H. diminuta* was confirmed by a coprological examination (Roepstorff and Nansen, 1998).

Lead exposure

For sub-acute Pb exposure, a modified methodology of Sures *et al.* (2002) was used in our study. According to the Sures study, Pb was administered orally to 12 hosts in the form of a lead acetate-trihydrate water solution (Analytika Ltd., Czech Republic) and Pb dosing was set at 7 μg per g of rat body weight. Twelve Pb doses were applied over a 6-week period. Lead acetate was chosen due to: (i) its high solubility and potential bioavailability and (ii) it is a standard Pb salt, used in experimental toxicology studies. Based on previous studies of Oyoo-Okoth *et al.* (2012), Sures and Siddall (2003) and Teodorova *et al.* (2003), 42 days of repetitive oral exposure conducted in our experiment should record the period with the most intensive Pb accumulation in both host and parasite tissues.

Tissue procedures

After Pb-exposure, experimental animals were sedated and euthanased with a T61 injection (Merck, Canada). Individual autopsies were carried out with Teflon instruments in order to obtain appropriate samples of tissue (liver, kidney, testes, duodenal wall, femoral muscle and bone) for Pb analyses. Subsequently, tapeworms (in total 94 individuals of *H. diminuta*) were obtained from duodenums of infected animals using the same instruments. All tissues were weighed, properly washed in double distilled water, placed into Petri dishes and stored at a temperature of −20 °C until they underwent chemical analysis.

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Table 2. Analytical data quality assessment

	Detection limit ($\mu g\ mL^{-1}$) (mean ± 3 S.D. of blanks)	CRM – BCR 12-02-01 determined value ($\mu g\ g^{-1}$) (mean ± S.D.)	CRM – BCR 12-02-01 certified value ($\mu g\ g^{-1}$) (mean ± S.D.)
Pb	0·21	0·746 ± 0·088	0·710 ± 0·08

S.D., standard deviation; CRM–BCR 12-02-01, Certified Reference material (BCR 12-02-01 Bovine liver).

Lead analysis

Prior to Pb-analysis, both rat and tapeworm tissue samples (except bones) were freeze-dried and microwave digested in an acid mixture using MWS-3+ microwave digestion system (Berghof Products+ Instruments, Germany). A minimum 200 mg of the dried sample was weighed into the Teflon digestion vessel DAP-60S. Subsequently, 2 mL of nitric acid 65%, p.a. ISO (Merck) and 3 mL H_2O_2 30%, TraceSelect (Fluka) were added. The mixture was shaken carefully, and after 1 h, the vessel was closed and heated in a microwave oven. The decomposition proceeded for 1 h in a temperature range of 100–190 °C. The digest obtained was transferred into a 50 mL silica beaker and evaporate into wet residue. The obtained residue was dissolved in 1·5% of HNO_3, and the dissolution was accelerated by sonication. Due to different structural characteristics, rat bones were mineralized according to the methodology of Mader *et al*. (1998). All obtained digests were subsequently transferred to probes and adjusted up to 14 mL with 1·5% of HNO_3. The atomic absorption spectrometry with electrothermal atomization (ET-AAS) technique (Varian AA 280Z, Australia) with a graphite tube atomizer GTA 120 and a PSD 120 programmable sample dispenser was used to determine C_{Pb} in all digested tissue and parasite samples of control groups as well as in the liver, muscle, testes and intestinal wall of exposed groups. The calibration curve for the measurement was prepared using standard Pb solution ASTASOL (Analytika, CR). The evaluation of C_{Pb} was carried out using a standard addition method, and ammonium dihydrogen phosphate GR (Merck) was used as a matrix modifier. The samples with elevated C_{Pb} (kidneys, bones and tapeworms) were analysed by using inductively coupled plasma optical emission spectrometry (ICP-OES, Varian VistaPro, Australia). Certified reference material BRC 12-02-01 (Bovine liver) was simultaneously analysed under the same conditions to assess the quality of analytical data (Table 2). Experimental data were corrected by the mean C_{Pb} in blanks (± 3 S.D. of blanks), which were prepared under the same conditions as tissues samples, and provides information about background levels of the trace element laboratory (detection limit). Samples of the tissues were analysed in two replicates, which were averaged for subsequent statistical processing. For detailed information on analytic procedures used in this experiment, see Table 2. C_{Pb} in the host and parasite tissues were determined as $\mu g\ g^{-1}$ of dry weight (DW) as well as $\mu g\ g^{-1}$ of fresh weight (FW).

C_{Pb} in host-parasite system

Based on determined C_{Pb} DW, the ratios of parasite C_{Pb} to host tissues C_{Pb} were expressed as bioconcentration factors (BCF) according to Sures *et al*. (1999*a*). Using the C_{Pb} FW of tapeworm and host tissues the real portion of ingested Pb accumulated in *H. diminuta* strobila was defined compared with that in host tissue. The kidney tissue was used for this comparison because: (i) concerning the host soft tissue, the kidneys reached the highest C_{Pb}, (ii) the FW of this organ was comparable to FW of tapeworms inside particular hosts.

Statistical procedure

The obtained datasets were tested using the Shapiro–Wilk's test for normal distribution. Since this requirement was not met (with the exception of muscle C_{Pb}), non-parametric procedures were performed using statistical software Statistica ver. 9 (StatSoft Inc., 2009). Based on Dixon's test 16 of the 156 values were considered outliers and they were excluded from further statistical processing. Differences in host tissue C_{Pb} between parasitized and non-parasitized animals were analysed using the Kruskal–Wallis test. For tapeworm tissues (only two groups), Mann–Whitney *U* and Wilcoxon tests were applied. At both exposure levels, the linear regression analyses were used to evaluate the relationship between: (i) C_{Pb} DW in particular host and tapeworm tissues; (ii) portion of Pb dose accumulated in tapeworm as well as in host tissue and the number of tapeworms as well as tapeworm FW per particular host. Differences were considered as significant at $P < 0{\cdot}05$ levels.

RESULTS

In the course of our parasitological/toxicological experiment, 156 samples of host and parasite tissues

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Table 3. Descriptive statistics of Pb concentrations in host and parasite tissues ($\mu g\,g^{-1}$ DW) expressed as median ± S.E. and (min-max) for particular group

Group Valid N	Liver 21	Kidney 21	Muscle 22	Bone 21	Intestine 23	Testes 21	Tapeworm 11
OO	$0{\cdot}08 \pm 0{\cdot}01^{TMt}$ (0·06–0·08)	$0{\cdot}13 \pm 0{\cdot}03^{OMt}$ (0·06–0·15)	$0{\cdot}04 \pm 0{\cdot}03$ (0·02–0·11)	$0{\cdot}09 \pm 0{\cdot}01^{OMt,TMt}$ (0·09–0·10)	$0{\cdot}07 \pm 0{\cdot}02$ (0·03–0·09)	$0{\cdot}06 \pm 0{\cdot}02$ (0·03–0·10)	X
TO	$0{\cdot}06 \pm 0{\cdot}03^{OMt,TMt}$ (0·01–0·09)	$0{\cdot}07 \pm 0{\cdot}02^{OMt,TMt}$ (0·05–0·10)	$0{\cdot}11 \pm 0{\cdot}02$ (0·08–0·12)	$0{\cdot}05 \pm 0{\cdot}01^{OMt,TMt}$ (0·05–0·06)	$0{\cdot}12 \pm 0{\cdot}04$ (0·10–0·20)	$0{\cdot}06 \pm 0{\cdot}01$ (0·05–0·09)	$0{\cdot}37 \pm 0{\cdot}05^{TMt}$ (0·26–0·41)
OMt	$0{\cdot}48 \pm 0{\cdot}12^{TO}$ (0·26–0·57)	$5{\cdot}26 \pm 0{\cdot}92^{OO,TO}$ (3·83–6·61)	$0{\cdot}09 \pm 0{\cdot}03$ (0·07–0·17)	$10{\cdot}56 \pm 1{\cdot}90^{OO,TO}$ (7·86–13·62)	$0{\cdot}26 \pm 0{\cdot}11$ (0·07–0·40)	$0{\cdot}20 \pm 0{\cdot}09$ (0·13–0·37)	X
TMt	$0{\cdot}58 \pm 0{\cdot}24^{OO,TO}$ (0·42–1·06)	$4{\cdot}98 \pm 1{\cdot}25^{TO}$ (2·80–6·44)	$0{\cdot}11 \pm 0{\cdot}06$ (0·04–0·18)	$9{\cdot}01 \pm 5{\cdot}00^{OO,TO}$ (2·54–14·06)	$0{\cdot}31 \pm 0{\cdot}21$ (0·04–0·62)	$0{\cdot}23 \pm 0{\cdot}11$ (0·06–0·54)	$23{\cdot}16 \pm 10{\cdot}85^{TO}$ (6·83–39·53)

OO-TMt–significant differences ($P < 0{\cdot}05$) compared to indexed groups (Kruskal-Wallis test with subsequent multiple comparison).
S.E., standard error; Min, minimum; Max, maximum; DW, dry weight; Valid N, number of variables in particular group; T, tapeworm *H. diminuta*; Mt, metal (lead acetate – trihydrate); O, no tapeworm/no exposure.

were obtained for Pb content analyses. In all Pb (lead acetate) treated animals, significantly higher ($P < 0{\cdot}05$) C_{Pb} were revealed when compared with those of both unexposed (OO and TO) groups. Muscle was the only tissue in which similar low values were found in both exposed and unexposed hosts. *Hymenolepis diminuta* showed 3- and 2·5-fold C_{Pb} DW (0·37 and 23·16) than those of the heaviest Pb-loaded host tissues (0·12 and 9·01) after both low and elevated exposure, respectively. Concerning rat tissues the highest C_{Pb} were detected in bones and kidney of both (OMt and TMt) Pb exposed groups (9·0 and $10{\cdot}6\,\mu g\,g^{-1}$ DW in bones; 5·3 and $5\,\mu g\,g^{-1}$ DW in kidneys, respectively). In all other analysed tissues C_{Pb} DW were below $1\,\mu g$ Pb g^{-1} DW. For detailed values, see Table 3.

Median values of tissue C_{Pb} differed among parasitized and non-parasitized animals. During normal Pb intake (only through commercial pellet feed), insignificantly lower Pb levels were detected in the livers, kidneys, testes and bones of parasitized animals; conversely, the muscles and intestinal walls of parasitized animals showed insignificantly higher Pb contents (Fig. 1). In Pb-exposed animals, a somewhat different situation was observed. In animals parasitized with *H. diminuta*, lower median values of C_{Pb} were determined only in the bones and kidneys. All other tissues (livers, muscles, testes, intestinal walls) of parasitized animals contained insignificantly more Pb than those of non-parasitized subjects (Fig. 2). However, at the same Pb exposure level, no differences between tissues C_{Pb} in parasitized and those of non-parasitized animals were statistically significant (Kruskal–Wallis test).

Hymenolepis diminuta tissues showed significantly ($P < 0{\cdot}01$, Mann–Whitney *U*-test) higher C_{Pb} after elevated Pb exposure than those in unexposed tapeworms (Fig. 3). Concerning both exposure levels, BCFs ranging from 2·6 (for bones) to 210 (for muscle) were determined. Generally, *H. diminuta* exhibits higher BCFs after elevated Pb intake (applies to muscle, liver, testes and the intestinal wall). Conversely, high Pb exposure caused a decrease in *H. diminuta* BCFs in comparison to the kidneys and bones. At a similar fresh weight tapeworm tissue accumulated significantly higher (Wilcoxon test, $P < 0{\cdot}001$) – 2·5 and 3-fold – portion (0·015 and 0·037) of ingested Pb compared with host kidney (0·006 and 0·013) after low and elevated Pb exposure, respectively (Table 4). After low Pb intake, the Pb dose portion accumulated in the *H. diminuta* tissue strongly correlate with number of tapeworms ($r^2 = 0{\cdot}98$, $P < 0{\cdot}001$) as well as with their fresh weight ($r^2 = 0{\cdot}83$, $P < 0{\cdot}023$) in particular rats. However, these relationships were not observed at high exposure level for either number of tapeworms ($r^2 = 0{\cdot}21$, $P = 0{\cdot}36$) or tapeworm FW ($r^2 = 0{\cdot}04$, $P = 0{\cdot}28$). Likewise, after both exposure levels, no significant correlations were found between Pb

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Table 4. Portion of Pb dose accumulated in parasite and host tissues (expressed as the median ± S.D.)

Group	No. of tapeworms[a]	Fresh weight[a] (g)		CPb ($\mu g\,g^{-1}$ FW)		Total Pb amount (μg)		Pb dose portion accumulated (%)	
		Tapeworm	Kidney	Tapeworm	Kidney	Tapeworm	Kidney	Tapeworm	Kidney
TO	6 ± 3	2·49 ± 0·77	3·32 ± 0·25	0·08 ± 0·04	0·02 ± 0·08	0·18 ± 0·39	0·08 ± 0·24	0·015 ± 0·03	0·006 ± 0·20
TMt	8 ± 4	3·34 ± 1·54	3·50 ± 0·27	4·04 ± 1·69	1·24 ± 1·69	13·0 ± 9·50	4·18 ± 1·30	0·037 ± 0·03	0·013 ± 0·01

[a] value per rat.
S.D., standard deviation; TO, parasitized rats with low Pb intake; TMt, parasitized rats with elevated Pb intake; FW, fresh weight.

portions accumulated in host kidney and either the tapeworm FW or tapeworm number per rat. Concerning changes in Pb levels (C_{Pb} DW) no significant correlations were detected between tapeworm and host tissues (detailed values are listed in Table 5).

DISCUSSION

Because the small intestine is simultaneously the predilection habitat of *H. diminuta* and the main site for Pb absorption after oral intake, tapeworms may interact with Pb in the duodenum. This interaction may affect the course of Pb in the host intestine as well as Pb absorption into the host body. Therefore, the aim of the present study was to determine the effect of tapeworm parasitization on C_{Pb} in host tissues. Our work draws on the modified experimental design carried out by Sures *et al*. (2002) and expands their work. Using a more sensitive analytical method together with a slight Pb-intake increase, we were able to improve the results of Sures *et al*. (2002) by determining C_{Pb} even in tissues with Pb levels that had been below detection limits in their study. By analysing C_{Pb} in six different host tissues after both low and elevated Pb exposure, this study provides the most comprehensive information concerning Pb toxicokinetics in a host-parasite system to date.

Six weeks of repetitive oral exposure conducted in our experiment should record the period with the most intensive Pb accumulation in both host (Teodorova *et al*. 2003) and parasite tissues (Sures and Siddall, 2003). After an oral intake, the majority of ingested Pb dose is finally stored in the bones, and high Pb concentrations are also usually found in the kidneys. However, this situation applies to GIH-free subjects. If parasitization is taken into account, predominant areas of Pb accumulation may change. Our results have confirmed this assumption only at low exposure level. During high Pb intake, the predominant tissues with Pb accumulation in parasitized and non-parasitized animals showed identical order (muscle < testes < intestinal wall < liver < kidney < bones), which is in compliance with results of previous toxicological studies. However, during low Pb intake, only non-parasitized animals exhibited typical Pb distribution in the organs, with higher Pb levels in the kidneys and bones. Conversely, the highest Pb concentrations were unexpectedly detected in the intestinal wall and muscle tissues of parasitized rats. These changes in Pb toxicokinetics within the host body may be caused by helminth parasitization that disrupts internal homeostasis, modulates immunological regulation of host organisms, and decreases levels of protective metallothioneins as described by Baudrimont and Montaudouin (2007). However, changes in Pb translocation in the organs of parasitized animals were negligible, and differences observed in our experiment cannot be generalized for all GIH-positive hosts.

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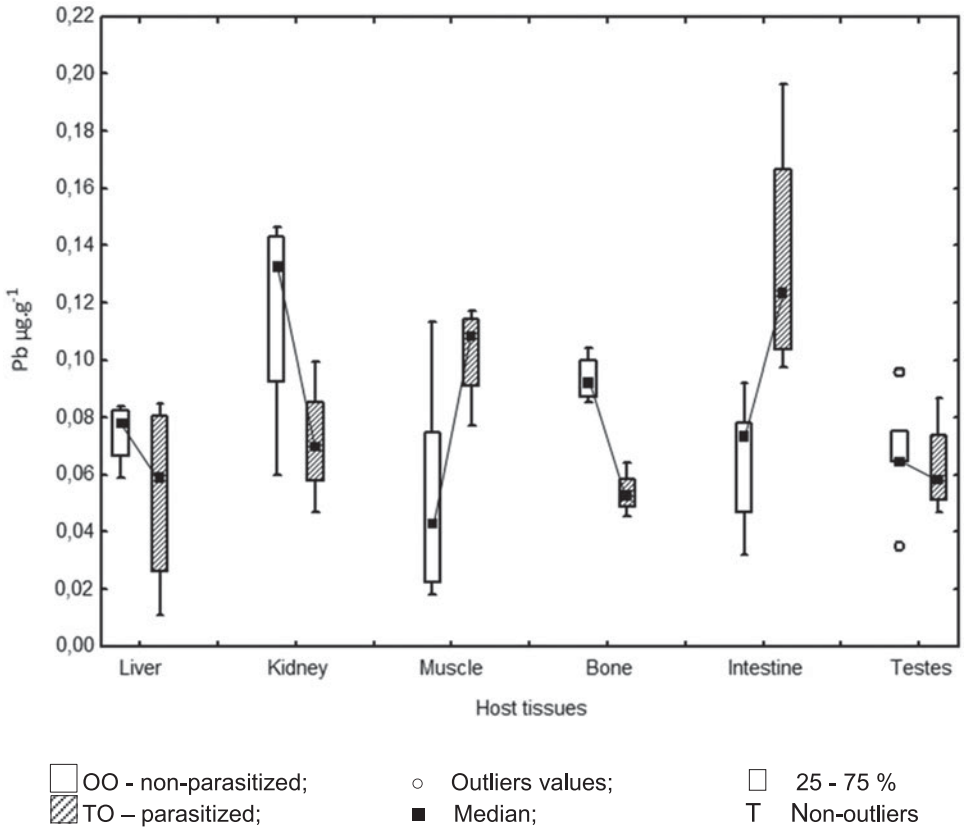

Fig. 1. Pb concentrations in host tissues after low Pb intake.

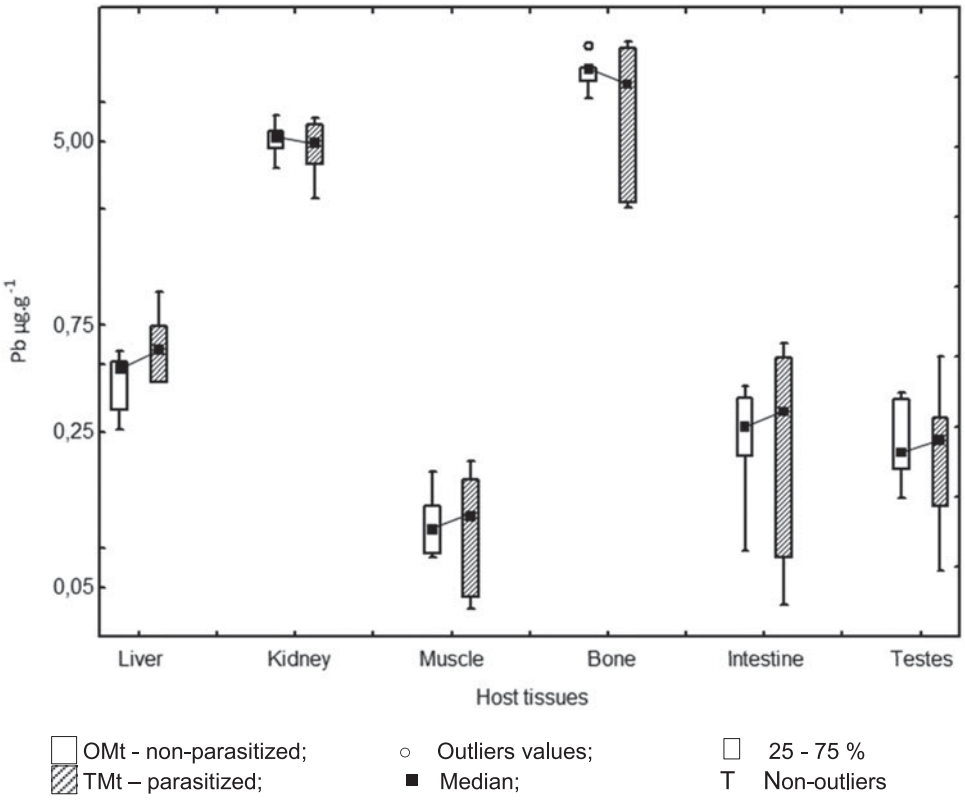

Fig. 2. Pb concentrations in host tissues after elevated Pb intake.

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Table 5. Correlation between Pb levels in tapeworm and host tissues

Exposure level	Relationship			r^2	*t*-test
Low	**Pb dose portion accumulated in tapeworm**	**–**	**Tapeworm No. per host**	**0·98**	***P*<0·001**
		–	**Tapeworm FW per host**	**0·83**	***P*<0·023**
	Pb dose portion accumulated in host kidney	–	Tapeworm No. per host	0·0004	*P* = 0·97
		–	Tapeworm FW per 1 host	0·09	*P* = 0·63
	Tapeworm C_{Pb} (DW)	–	Host liver C_{Pb} (DW)	0·08	*P* = 0·65
		–	Host kidney C_{Pb} (DW)	0·18	*P* = 0·47
		–	Host muscle C_{Pb} (DW)	0·002	*P* = 0·94
		–	Host bone C_{Pb} (DW)	0·15	*P* = 0·52
		–	Host intestine C_{Pb} (DW)	0·53	*P* = 0·17
		–	Host testes C_{Pb} (DW)	0·09	*P* = 0·63
Elevated	Pb dose portion accumulated in tapeworm	–	Tapeworm No. per host	0·21	*P* = 0·36
		–	Tapeworm FW per host	0·04	*P* = 0·28
	Pb dose portion accumulated in host kidney	–	Tapeworm No. per host	0·004	*P* = 0·90
		–	Tapeworm FW per host	0·31	*P* = 0·33
	Tapeworm C_{Pb} (DW)	–	Host liver C_{Pb} (DW)	0·09	*P* = 0·56
		–	Host kidney C_{Pb} (DW)	0·18	*P* = 0·42
		–	Host muscle C_{Pb} (DW)	0·13	*P* = 0·47
		–	Host bone C_{Pb} (DW)	0·08	*P* = 0·59
		–	Host intestine C_{Pb} (DW)	0·05	*P* = 0·67
		–	Host testes C_{Pb} (DW)	0·18	*P* = 0·40

– significant correlations in bold.
C_{Pb}, Pb concentration; DW, dry weight; FW, fresh weight.

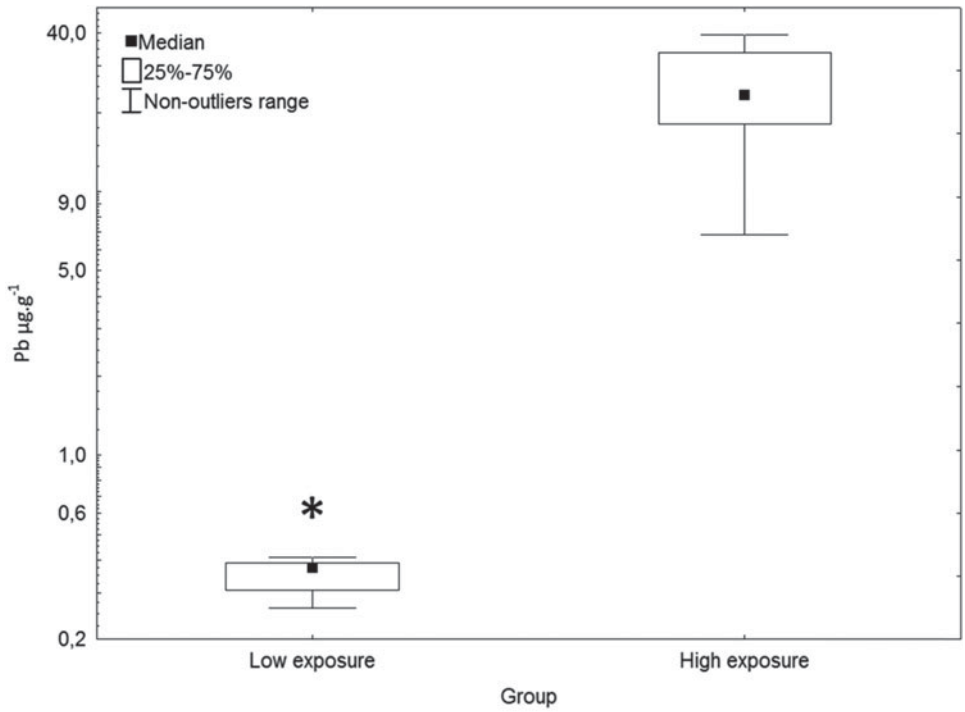

Fig. 3. Pb concentrations in *Hymenolepis diminuta* after low and elevated host exposure. *, significant ($P<0.01$) differences (Mann-Whitney *U*-test).

In accordance with numerous previous studies (Baruš *et al*. 2000; Sures *et al*. 2002, 2003*b*; Torres *et al*. 2004; Malek *et al*. 2007), the tapeworm tissues from our study displayed the highest C_{Pb} concerning the entire host-parasite system (after both low and elevated Pb exposures). Generally, with the similar fresh weight, *H. diminuta* accumulates up to 3-fold portion of ingested Pb dose compared with that of the host most loaded soft tissue (kidney) at both exposure levels. Therefore, our research clearly confirmed hyper-accumulative properties of *H. diminuta* for Pb, and the Hymenolepid tapeworm could serve as a suitable accumulative indicator of environmental pollution as introduced by Torres *et al*. (2011) and

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Table 6. Differences in tapeworm bioconcentration factors (BCFs) between low and high Pb exposure – comparison to previous studies

Author	Host	Parasite	Exposure	Parasite BCF in comparison to host tissues					
				Kidney	Liver	Muscle	Intestine	Bone	Testes
Our experiment	*Rattus norvegicus*	*Hymenolepis diminuta*	Low	5·3	6·2	3·4	3·1	7·4	6·2
			High	4·7	40	210	75	2·6	100
Sures *et al.* (2003*b*)	*Rattus norvegicus*	*Hymenolepis diminuta*	Low	6	29		36		
			High	11	87		87		
Torres *et al.* (2006)	*Apodemus sylvaticus*	*Skrjabinotaenia lobata*	Low	5·6	33·1	52·4			
			High	8·5	53·2	81·4			
Jankovska *et al.* (2010*a*)	*Ovis aries*	*Moniezia expanza*	Low	0·2	0·3	0·9			
			High	4	5	458			

Sures *et al.* (2002, 2003*b*). Unlike field studies, we were able to determine the exact Pb-dose portion stored in fresh tapeworm tissue which was 0·015 and 0·037% after low and elevated exposure, respectively. Pb dose portion accumulated in *H. diminuta* tissue after low Pb intake strongly correlated with tapeworm biomass (number of tapeworms and fresh weight) in particular host. Similar relationship concerning intensity of tapeworm (*Oochoristica tuberculata*) infection and their Pb levels were detected by Soliman (2012) in lizards. Contrary to this, no significant correlation between parasite biomass and Pb levels in host kidney was revealed in our experiment. This finding is consistent with the results of Nachev *et al.* (2010) and Oyoo-Okoth *et al.* (2010) who found no significant role both of Acanthocephalans and tapeworm larvae abundances for Pb body burden in aquatic host-parasite systems. As far we know, our study determined this relationship in mammalian/cestodes model for the first time. We also defined correlation coefficients for Pb concentration in parasite and rat tissue. However, according to our results, changes in tapeworm Pb levels were unrelated to those of host tissues. The opposite trend was found by Nachev *et al.* (2013) between nematode larvae (Eustrongylides sp.) and their fish host. Such differences can be likely explained by different localization of these parasitic species as well as by differences in heavy metals uptake and metabolism between adult cestodes and nematode larvae.

The higher BCFs in Pb-exposed tapeworms observed in our experiment are consistent with the previous field study results of Sures *et al.* (2003*b*) and Torres *et al.* (2006), which showed elevated (2–3-fold) tapeworm BCFs in rodents living in Pb-polluted areas. A detailed comparison of our results to those of previous works is given in Table 6. Conversely, Jankovska *et al.* (2010*a*), who conducted short-term experimental oral exposure in sheep, recorded much more pronounced (20–500-fold) differences between BCFs of exposed animals and those of unexposed animals. In our sub-acute study, the coefficients between BCFs in high- and low-exposed animals ranged from 6 (for liver) to 60 (for muscle). Only bones and kidney did not exhibit a dose-dependent pattern of BCFs. Such differences in BCFs determined after short- and long-term Pb exposure complies with previous studies of Sures and Siddall (2003) and Oyoo-Okoth *et al.* (2012), which indicated that tapeworms are able to accumulate heavy metals much more rapidly than are host soft tissues, and Pb levels in tapeworm tissue reach steady-state concentration in 4 weeks post-exposure. Subsequently, differences between C_{Pb} of parasites and those of host tissues gradually become less evident during chronic exposure and BCFs usually decreases according to these authors.

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With regards to host tissues, C_{Pb} in both parasitized and non-parasitized rats were similar during low Pb intake. Conversely, during elevated Pb treatment C_{Pb} increased 50-fold and more than 100-fold in rat kidneys and bones, respectively. The kidneys and bones, which are typically predominant areas of high Pb accumulation, were also the only two tissues whose final Pb levels (median C_{Pb}) were lower in parasitized animals than those of non-infected subjects during both low and elevated Pb exposure. Variable results of analysed other host tissues showed that the tapeworm effect on final C_{Pb} in the host body seems to be very complex and is almost impossible to describe in a simplistic way. Such a phenomenon has also been indicated in previous studies of Jankovska *et al*. (2008, 2010*a*, *b*) and Sures *et al*. (2002), which provided partially inconsistent results concerning the effect of GIH parasitization on heavy metal accumulation in host tissues. In our study, C_{Pb} in the kidneys of parasitized rats were only insignificantly lower than those of their non-parasitized conspecifics; this observation complies with the results of Sures *et al*. (2002). However, Sures study did not provide values concerning C_{Pb} in other host tissues. Data obtained from the kidneys and livers in Pb-treated animals in our experiment are also similar to those of the field screening by Jankovska *et al*. (2010*b*), in which parasitized foxes from a polluted area exhibited lower C_{Pb} in the kidneys and higher C_{Pb} in the liver than those of their parasite-free conspecifics. Notwithstanding, tapeworm-positive rodents from the same area exhibited lower C_{Pb} in both the liver and kidneys than did the uninfected animals (Jankovska *et al*. 2008). As with our results, differences between C_{Pb} in the organs of tapeworm-positive and -negative animals were always statistically insignificant. Only one terrestrial experimental study (Jankovska *et al*. 2010*a*), which was conducted on tapeworm-infected and non-infected sheep, detected significantly reduced C_{Pb} in both the liver and kidneys of parasitized animals. However, these results of short-term Pb intake were not confirmed by our sub-chronic exposure under standardized conditions.

Moreover, our results do not fully support the prior hypotheses of Azmat *et al*. (2008) and Jankovska *et al*. (2012), which state that intestinal helminths are able to prevent their host from absorbing ingested heavy metals because some host tissues of parasitized rats in our experiment exhibited higher C_{Pb} that those of their non-infected conspecifics. Although tapeworm tissue accumulates large amounts of Pb, just the presence of tapeworms in the duodenum may paradoxically facilitate Pb absorption in the host body. This is mainly because acidic products of *H. diminuta* glucose metabolism can decrease host intestinal pH levels to under 6 (Mettrick, 1971), which may increase Pb bioavailability. Moreover, tapeworm strobila partially clogs the intestinal lumen and slows down chyme movement through the intestine (Dwinell *et al*. 1997); this process enables higher Pb absorption by the host body. Finally, tapeworms take up certain essential elements (primarily Ca, Mg and Fe) from the chyme into their tissues, and this uptake could free up transport routes for Pb absorption because these elements use the same transmission paths (through the intestinal epithelium) as does Pb (Barton *et al*. 1978*a*, *b*; Morrison and Quarterman, 1987). In addition parasites may also cause an increase in host tissue heavy metal levels, by disrupting their risk elements regulation system (Oyoo-Okoth *et al*. 2012). Our study confirms this assumption, especially within the course of elevated Pb exposure for all tissues with low (less than 1 μg Pb g^{-1} DW) final Pb concentrations (liver, muscle, intestinal wall and testes). Moreover, the intestinal wall, through which the absorbed Pb passes, displayed higher Pb concentrations in parasitized rats when compared with those of their parasite-free conspecifics at both exposure levels of our experiment. Because the high C_{Pb} in tapeworm tissues were not associated with simultaneous significant decrease in host tissues C_{Pb}, it can be assumed that tapeworms are most likely to take up bile-bound Pb, which is primarily absorbed into the host body and secondarily excreted into the duodenum via the hepatic cycle. A similar process has been demonstrated in other GIH by Sures *et al*. (1998) and Sures and Siddall (1999). Thus, in our opinion tapeworms are not able to actively eliminate pollutants from host tissues. However, metal absorption in helminthes is yet to be fully understood because the essentiality of Pb has never been demonstrated in any animal. To verify potential mechanism of bile-bound Pb in tapeworms, more detailed experiments, similar to that of Sures and Siddall (1999) conducted on Acanthocephalans should be carried out in adult tapeworms.

Based on our results, the misinterpretation of environmental pollution biomonitoring data obtained from parasitized subjects, presented by Sures *et al*. (2003*a*), should not be a relevant factor, because C_{Pb} did not differ significantly between parasitized and non-parasitized animals. A somewhat higher risk can be seen in the bias of biomonitoring data from unpolluted areas, because parasitization may affect the predominant areas of Pb accumulation in host tissues during low Pb exposure. To obtain more accurate results, specific research should be conducted at various levels of exposure.

This study experimentally verified the effect of tapeworm parasitization on Pb accumulation in host tissues. Our results confirmed hyperaccumulative ability of *H. diminuta* for Pb. This fact predetermines this species as a suitable subject for the bioindication of environmental pollution. Concerning the same exposure level, no significant changes were detected in Pb tissue levels between

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parasitized and non-parasitized subjects. Based on these data we can assume that tapeworms are unable to protect their host from elevated Pb accumulation. The kidney and bones, which are predominant areas of high Pb accumulation, were the only two tissues in which decreases in C_{Pb} were observed in parasitized animals after periods of both low and elevated Pb exposure. At low exposure levels, tapeworm parasitization slightly changed the Pb toxicokinetics in the host body. However, the above-mentioned changes were statistically insignificant. Therefore, they cannot be applied to all tapeworm-infected hosts.

The results of this study provide insight into interactions between GIH parasitization and host oral exposure to environmental pollutants and indicate that *H. diminuta* most likely takes up Pb, which was primarily absorbed into the body and subsequently excreted back into the small intestine via the hepatic cycle. It is necessary to acknowledge that data obtained in our study are not as clear as expected, suggesting that this is a complex issue. Therefore, further research on this topic should be carried out. For example, the determination of C_{Pb} in host blood and bile during the exposure period will help to clarify possible changes in Pb intestinal absorption, Pb toxicokinetics in the host body, and Pb excretion through the hepatic cycle. The ability of adult tapeworms to take up bile-bound Pb should be verified by *in vitro* cultivation in a medium enriched with bile-salt and Pb compounds.

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ETHICAL STANDARDS – EXPERIMENTAL ANIMALS

All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on Protection of Animals against Cruelty and EC Directive 86/609/EEC. Animal care was supervised by an authorized person: Zuzana Čadková, holder of the Central Commission for Animal Welfare Certificate No. CZ 00354.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. All authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal.

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