Heavy metals in two host-parasite systems: tapeworm vs. fish

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Abstract

The tissue of two tapeworm species (*Ligula intestinalis* and *Bathybothrium rectangulum*) and body muscles of their fish host species were analyzed for heavy metal concentrations by standard methods using atomic absorption spectrometry. Regarding the values of accumulation ratio, the *L. intestinalis* accumulated $12.5-18.9 \times$ more lead, $2.3-3 \times$ more cadmium, and $4.4-14.1 \times$ more chrome, compared to respective metal concentrations in muscles of cyprinid intermediate fish hosts. The gravid strobila biomass of the *B. rectangulum* accumulated $2.2 \times$ more lead, $1.2 \times$ more nickel, and $2.3 \times$ more chrome compared with the respective concentrations in the muscles of the barbel *Barbus*. Metal concentrations in the muscles of unifieded fish and by tapeworm infected barbels showed that the unifieded individuals exhibited $1.4 \times$ more lead, $1.6 \times$ more nickel and $1.7 \times$ more chrome than the infected ones. Our study suggests that parasites are a useful bioindicator when evaluating environmental pollution of aquatic ecosystems by heavy metals.

Ligula, Bathybothrium, cyprinid fishes

The accumulation and concentration of heavy metals in aquatic organisms (especially in fish) has been investigated in the Czech Republic on a long-term basis (see review by Peňáz et al. 1980, 2002; Svobodová and Hejtmánek 1985; Svobodová et al. 1996, 1999; Spurný et al. 2009; Valová et al. 2010). Interest in the study of the heavy metal bioaccumulation potential in the strobila of adult tapeworms parasitizing in the intestines of both freshwater and marine fishes increased in the last decade of the past century (Riggs et al. 1987; Sures and Taraschewski 1995; Taraschewski and Sures 1996; Sures et al. 1997ab, 1999; Turčeková and Hanzelová 1996, 1999). Previous studies were focussed also on larval stages of parasites and heavy metal concentrations were analyzed in plerocercoids of the tapeworm *Ligula intestinalis* (L.) parasitizing in the body cavity of cyprinids (Gabrashanska and Nedeva 1996; Svobodová et al. 1996; Tenora et al. 1997, 2000; Baruš et al. 1999, 2001). Pascoe and Cram (1977) studied the accumulation of cadmium in plerocercoids of a related tapeworm species Schistocephalus solidus (Müller, 1776). Studies published until now have documented also the theoretical and practical problems of heavy metal concentrations in the strobila of tapeworms and in the tissues (mostly muscles and liver) of their intermediate and/or definite fish hosts. The aim of this study was to analyze heavy metal concentrations in two different parasite species and their host species from the natural aquatic environment of the Czech Republic.

Materials and Methods

Several host-parasite systems with the following sample size (n) were analyzed. First, three host species, i.e. common bream *Abramis brama* (L.) (n = 10), white bream *Blicca bjoerkna* (L.) (n = 15) and roach *Rutilus rutilus* (L.) (n = 10) parasitized by *Ligula intestinalis* were studied. The plerocercoids of this tapeworm and samples of fish muscles (of age 3+ and older) from 3 localities (i.e. Mostiště, Dalešice and Nové Mlýny reservoirs) were sampled in 1992-1995 and studied. Next, barbel *Barbus barbus* (L.) (n = 7) infected by *Bathybothrium rectangulum* (Bloch, 1782) was studied in the same season of 2004. The tapeworm strobila and muscles of parasitized fish (of age 4+ and 5+) from the barbel zone in the fishing region of Jihlava River were analyzed.

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Phone: +420 543 422 533 E-mail: vetesnik@ivb.cz http://actavet.vfu.cz/ In addition, fish species non-infected by this parasite, of the same age and from the same locality (n = 5), and samples of macrophytes - water crowfoot (*Batrachium fluitans* Lam.) (n = 3) were analyzed.

Heavy metal concentrations (lead - Pb, cadmium - Cd, chrome – Cr and nickel - Ni) were determined by means of atomic absorption spectrometry (AS) with the apparatus GBC – 932 A (USA) (for more details see Baruš et al. 1999, 2001). The contents of heavy metals were expressed in mg·kg⁻¹ of 100% dry matter. The coefficient R (ratio) was also calculated to evaluate the efficiency and differences in heavy metals concentrations in the parasite biomass and muscles of hosts as the proportion of the parasite and host heavy metals concentrations (following Sures et al. 1999).

Results

Heavy metal concentrations in host-parasite systems including *Ligula intestinalis* and their cyprinid fish hosts are shown in Table 1. Concentrations of the detected heavy metals (Pb, Cd, Cr) accumulated in the biomass of *L. intestinalis* plerocercoids were higher than those in the muscles of three intermediate host fish (*A. brama*, *B. bjoerkna* and *R. rutilus*). According to the R-values, Pb is the most efficiently accumulated element, followed by Cr and Cd (see Table 1). When comparing three species analyzed in this study, the lowest R-values were found in roach and the highest ones in common bream. Similar R-values of Cd were found in three host species.

Table 1. Heavy metal (Pb, Cd and Cr) concentrations (mg·kg⁻¹ in 100% dry matter) in plerocercoids of tapeworm *Ligula intestinalis* and in muscles of parasitized fish (*Abramis brama, Blicca bjoerkna* and *Rutilus rutilus*; according to Tenora et al. 2000, supplemented)

	Intermediate host									
	Abramis brama			Blicca bjoerkna				Rutilus rutilus		
Heavy metals	parasite	muscle	R	parasite	muscle	R	parasite	muscle	R	
Pb	3.40 ± 0.05	0.18 ± 0.05	18.89	3.20 ± 0.04	0.09 ± 0.04	16.84	4.51 ± 0.32	0.36 ± 0.02	12.53	
Cd	0.21 ± 0.08	0.07 ± 0.02	3.00	0.21 ± 0.09	0.09 ± 0.01	2.33	0.31 ± 0.09	0.11 ± 0.01	2.82	
Cr	1.13 ± 0.08	0.08 ± 0.01	14.13	1.14 ± 0.07	0.10 ± 0.01	11.40	3.16 ± 0.09	0.71 ± 0.02	4.45	
Dry matter (%)	20.37	29.61	-	21.15	28.12	-	18.16	27.53	-	

Heavy metal concentrations in host-parasite system including *Bathybothrium rectangulum* and barbel are shown in Table 2. Of the heavy metals evaluated, higher concentrations of Pb, Ni and Cr were regularly found in the strobila of adult tapeworms compared to muscles of parasitized fish. Cadmium did not show any tendency towards higher accumulation in parasites and similar Cd concentrations were found in the parasite's strobila and in muscles of both the parasitized and non-parasitized fish. Based on the R values, accumulation effect of Pb, Ni and Cr are relatively balanced (see Table 2). No significant accumulation of Cd was found in the parasite's strobila ($R \le 1$). Heavy metal concentrations in muscles of parasitized and non-parasitized barbel were different; the concentrations of Pb, Ni and Cr in non-parasitized individuals were higher than those in parasitized individuals, whereas the concentration of Cd in both groups of individuals was nearly identical. The concentrations of Pb and Cr in muscles of non-parasitized fish were slightly lower than the concentrations of these heavy metals in the tapeworm strobila. On the contrary, the Ni concentration in muscles of non-parasitized fish slightly prevailed over that in the tapeworm strobila.

The concentrations of Cd, Ni and Cr in the river water crowfoot (*B. fluitans*) were evidently higher compared to the tapeworms and both parasitized and non-parasitized fish. Concentrations of Pb in the plant and fish were similar but slightly lower than that of the parasite strobila (Table 2).

Table 2. Heavy metals (Pb, Cd, Ni, Cr) concentrations ($mg \cdot kg^{-1}$ in 100% dry matter) in strobila of adult tapeworms *Bathybothrium rectangulum*, in muscles of parasitized and non-parasitized *Barbus barbus* and in aquatic plant, river water crowfoot (*Batrachium fluitans*)

Heavy metals	Parasite	Host-	muscle	Aquatic	R	
ficavy inclais	1 arasite	parasitized	non-parasitized	plant		
Pb	1.31 ± 0.82	0.60 ± 0.08	0.82 ± 0.08	0.81 ± 0.10	2.22	
Cd	0.21 ± 0.04	0.23 ± 0.05	0.23 ± 0.04	8.80 ± 0.18	0.88	
Ni	1.05 ± 0.04	0.86 ± 0.09	1.35 ± 0.10	3.33 ± 0.13	1.23	
Cr	1.87 ± 0.05	0.82 ± 0.06	1.39 ± 0.06	12.68 ± 0.22	2.29	
Dry matter (%)	19.85	28.98	30.80	11.23	-	

Discussion

The bioaccumulation potential of parasites, as a result of competition for chemical elements (including heavy metals), represents a valuable tool to evaluate functions of the host-parasite systems (Sures et al. 1999). Such information is available for larval stages of tapeworm species parasitizing as invasive plerocercoids in the body cavity of intermediate host fishes. Pascoe and Cram (1977) found lower Cd concentrations in plerocercoids of the tapeworm S. solidus compared to muscles of its intermediate host i.e. the three-spined stickleback Gasterosteus aculeatus (L.). Gabrashanska and Nedeva (1996) observed regularly higher concentrations of Cu, Cr and Zn (without giving more accurate data) in plerocercoids of L. intestinalis than in muscles of the bleak Alburnus alburnus (L.). Our study showed that plerocercoids of L. intestinalis exhibit a specifically different effective bioaccumulation potential for some heavy metals (Pb, Cd and Cr). Similar findings were previously demonstrated by Tenora et al. (2000) and Baruš et al. (2001). The variability in concentrations of these heavy metals in tapeworm plerocercoids reflects, in our opinion, both the age of the intermediate host fish and the age of plerocercoids, i.e., the time of heavy metal exposition of hosts and parasites in a differently contaminated aquatic environment. This hypothesis is supported by the fact that concentrations of these heavy metals are regularly and significantly higher in older tapeworm plerocercoids (Baruš et al. 2001; Baruš and Prokeš 2002). Similar results were also shown on bioaccumulation and concentrations of some heavy metals in the strobila of adult tapeworms parasitizing in the intestine of definitive fish hosts. Riggs et al. (1987) found that the concentration of selenium (Se) in the strobila of tapeworm *Bothriocephalus acheilognathi* (Yamaguti, 1934) is $8 \times$ higher than in the muscles of parasitized cyprinid fishes. Turčeková and Hanzelová (1996) stated the Pb, Cd, Zn, Cu and As concentrations in the strobila of tapeworm *Proteocephalus percae* (Müller, 1780) were 200 to $350 \times$ higher than in the muscles of perch *Perca* fluviatilis (L.). Grabrashanska and Nedeva (1996) ascertained higher Cu, Cr and Zn concentrations in the tapeworm *Carvophyllaeus brachycollis* (Janiszewska, 1951) compared to their concentrations in muscles of vimba bream Vimba vimba (L.). Sures et al. (1997a) analyzed accumulation of Pb and Cd in two tapeworm species, namely Monobothrium wageneri (Nybelin, 1922) parasitizing tench Tinca tinca (L.) and Bothriocephalus scorpii (Müller, 1776) from turbot Scophthalmus maximus (L.). They found that tapeworm *M. wageneri* accumulated 75 \times more Pb and 34 \times more Cd against concentrations found in muscles of the host. In contrast to this observation, an efficient bioaccumulation of Pb was not found in the tapeworm *B. scorpii* (R = 1-2.25), however, it was considerably higher for Cd (R = 35-60). The relation between heavy metals concentration and the age of parasites (i.e. time of exposition) was documented by Riggs et al. (1987) and Sures et al. (1997a) using comparative analysis of anterior

parts of tapeworms and gravid proglottids of *B. acheilognathi* and *B. scorpii*. The analyzed Pb, Se and Cd were characterized by significantly higher concentrations in gravid proglottids. According to our results, bioaccumulation of heavy metals (Pb, Cr and Ni) in the strobila of *B. rectangulum* is relatively low and comparable with data presented for Pb by Sures et al. (1997a). The accumulation of Cd in the strobila of *B. rectangulum* investigated in our study was the lowest compared to all of the above studies. It means that the Cd concentration in parasites does not markedly differ from that in muscles of the hosts.

All of this suggests that the bioaccumulation capacity for some heavy metals in tapeworms (plerocercoids and adult worms) parasitizing in the body cavity and intestines of host fishes is expressed regularly by higher concentrations in the strobila of tapeworms than in muscles of parasitized hosts. Accumulation of Cd and Pb, on the contrary, is manifested less markedly (R < 2), with few exceptions (*P. percae* and *B. scorpii*). So far it could not be stated unambiguously if this is connected with the influence of external environment (concentration of heavy metals, time of exposition, nutrition of the fish) or with the species-specific accumulation ability of tapeworms.

The parasite's life span (determining the time of exposition in the host) is an important factor in adult tapeworms parasitizing in the intestine of the fish. It is relatively short (one year) compared with a rather long exposition of larval stages parasitizing in cavities and tissues of intermediate host fishes. The function of host-parasite system such is the cyprinid fish and the plerocercoids of *L. intestinalis* is comparable with the life span of intermediate host (Baruš and Prokeš 1995).

Gabrashanska and Nedeva (1996) pointed out an interesting aspect of competition for chemical elements in the host-parasite systems when they found that concentrations of Cu and Zn in muscles of fishes (*V. vimba, A. alburnus*) parasitized by tapeworms (*C. brachycollis, L. intestinalis*) were lower than those in muscles of non-parasitized fish. Turčeková and Hanzelová (1999) documented such differences for Cd and As in muscles of non-parasitized fish and with tapeworm *P. percae* parasitized muscles of perch. Concentrations of Cd and As were up to $5 \times$ higher in the non-parasitized than in parasitized fish. On the other hand, concentrations of Pb in muscles of non-parasitized fish were lower than in parasitized fish. The heavy metals concentrations ascertained during our study in the non-parasitized fish and with the tapeworm *B. rectangulum* parasitized barbel are somewhat different: Pb in muscles of non-parasitized fish, whereas Cd concentration was similar in both fish groups.

We consider as very likely the presumption by Turčeková and Hanzelová (1999) that tapeworms may decrease the burden of some heavy metals in their hosts and thus prevent their intoxication. Fish parasites (especially Acanthocephala and Cestoda) are actually considered as useful bioindicator of polluted aquatic environment (Turčeková and Hanzelová 1999; Sures et al. 1997ab, 1999; Sures and Taraschewski 1995). *Barbus barbus* is also considered a very appropriate indicator to asses the pollution of aquatic environment with Hg (Peňáz et al. 1979, 1980; Svobodová et al. 1996; Spurný et al. 2009). In our study, concentration of two heavy metals (Ni and Cr) determined in the water crowfoot (*B. fluitans*) confirmed a high bioaccumulation potential of aquatic macrophytes (see Peňáz et al. 1980; Svobodová et al. 1996).

Heavy metal concentrations in fish (definite or intermediate hosts of tapeworms) are measured in certain organs, e.g. muscles or liver, whereas the heavy metals in the body of tapeworms reflect their concentration in the entire parasite's organism. In this respect, the knowledge of heavy metals in parasites may serve as a convenient and additional bioindicator for evaluating environmental pollution of aquatic ecosystems.

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References

- Baruš V, Prokeš M 1995: Length-weight relationship of *Ligula intestinalis* plerocercoids in adult *Blicca bjoerkna* and discussion on estimation of parasite age. Appl Parasitol **36**: 192-199
- Baruš V, Prokeš M 2002: Length and weight of *Ligula intestinalis* plerocercoids (Cestoda) parasitizing cyprinid fishes (Cyprinidae): a comparative analysis. Helminthologia **39**: 29-34
- Baruš V, Tenora F, Kráčmar S, Dvořáček J 1999: Contents of several inorganic substances in European eel infected and uninfected by *Anguillicola crassus* (Nematoda). Dis Aquat Org **37**: 135-137
- Baruš V, Tenora F, Kráčmar S, Prokeš M 2001: Accumulation of heavy metals in plerocercoids of *Ligula intestinalis* (Pseudophyllidea) of different age. Helminthologia **38**: 29-33
- Gabrashanska M, Nedeva I 1996: Content of heavy metals in the system fish-cestodes. Parassitologia 38, p. 58
- Pascoe D, Cram P 1977: The effect of parasitism on the toxicity of cadmium to the three-spines stickleback, Gasterosteus aculeatus L. J Fish Biol 10: 467-472
- Peňáz M, Baruš V, Prokeš M 2002: Concentrations of heavy metals and changes in concentrations of the mercury in tissues of the barbel (*Barbus barbus*) from the River Jihlava (in Czech with English summary). Proc. of the Czech Conference of Ichthyology, Brno 2002, pp. 253-258
- Peňáz M, Svobodová Z, Hejtmánek M, Trnková J 1979: Mercury contents in muscles of fishes from the Jihlava River. Folia Zool 28: 171-176
- Peňáz M, Svobodová Z, Hejtmánek M, Trnková J, Wohlgemuth E 1980: Mercury contents in the basic components of Jihlava river ecosystem (in Czech). Bulletin VÚRH Vodňany 1: 24-30
- Riggs M R, Lemly A D, Esch G W 1987: The growth, biomass and fecundity of *Bothriocephalus acheilognathi* in a North Carolina reservoir. J Parasitol **73**: 893-900
- Spurný P, Mareš J, Hedvábný J, Sukop I 2009: Residual metal contamination of the ecosystem in the lower courses of the Jihlava River. Acta Vet Brno 78: 525-534
- Sures B, Siddal R, Taraschewski H 1999: Parasites as accumulation indicators of heavy metal pollution. Parasitol Today 15: 16-21
- Sures B, Taraschewski H 1995: Helminths of fish: reliable indicators of heavy metal pollution in aquatic ecosystems? Bull Scand Soc Parasit 5: 73
- Sures B, Taraschewski H, Rokicki J 1997a: Lead and cadmium content of two cestodes, Monobothrium wageneri and Bothriocephalus scorpii, and their fish host. Parasitol Res 83: 618-623
- Sures B, Taraschewski H, Siddal R 1997b: Heavy metal concentrations in adult acanthocephalans and cestodes compared to their fish host and to established free living bioindicators. Parassitologia **39**: 213-218
- Svobodová Z, Hejtmánek, M 1985: Total mercury content in the components of running water, reservoir and pond ecosystems in Czechoslovakia. Symp. Biologica Hungarica 29: 171-177
- Svobodová Z, Dušek L, Hejtmánek M, Vykusová B, Šmíd R 1999: Bioaccumulation of mercury in various fish species from Orlík and Kamýk water reservoirs in the Czech Republic. Ecotox Environ Safe 43: 231-240
- Svobodová Z, Máchová J, Vykusová B, Piačka V 1996: The heavy metals in water ecosystems (in Czech). Edice metodik, č. 49. VÚRH Vodňany, pp. 1-19
- Taraschewski H, Sures B 1996: Heavy metal concentrations in parasites compared to their fish hosts: bioconcentration by acanthocephalans and cestodes. Parassitologia **38**: 65
- Tenora F, Kráčmar S, Baruš V, Dvořáček J 1997: Some anorganic substances in plerocercoids of *Ligula intestinalis* (Pseudophyllidea). Acta Univ Agric Silvic Mendelianae Brunensis **95**: 23-30
- Tenora F, Kráčmar S, Baruš V, Dvořáček J 2000: Concentrations of some heavy metals in *Ligula intestinalis* plerocercoids (Cestoda) and *Philometra ovata* (Nematoda) compared to some their host (Osteichthyes). Helminthologia 37: 15-18
- Turčeková L, Hanzelová V 1996: Concentration of heavy metals in cestode Proteocephalus percae, parasite of perch. Helminthologia 33: 162-163
- Turčeková L, Hanzelová V 1999: Concentrations of Cd, As and Pb in non-infected and infected Perca fluviatilis with Proteocephalus percae. Helminthologia 36 (Suppl.): p. 31
- Valová Z, Jurajda P, Janáč M, Bernardová I, Hudcová D 2010: Spatiotemporal trends of heavy metal concentrations in fish of the River Morava (Danube basin). J Environ Sci Heal A **45**: 1892-1899