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Concentrations of selected heavy metals in *Ligula intestinalis* L., 1758 plerocercoids (Cestoda) compared to it host's (*Tinca tinca* L., 1758) organs from Beyşehir Lake (Turkey)

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Summary

The accumulation of heavy metals (Cu, Fe, Zn and Mn) was measured by atomic absorption spectrophotometry in some organs of tench (*Tinca tinca* L., 1758) and tissues of its parasite collected from Beyşehir Lake, and compared with the data from sediments and water. The Cu levels in *L. intestinalis* L., 1758 plerocercoids were 1.69 times, respectively, higher than those in fish gill. Zn level in *L. intestinalis* plerocercoids was 2.13 times higher than those in fish liver, was 5.53 times higher than that of the muscle and 3.33 times higher than that in the gill. Significant negative (for Mn) correlation was found between the quantity of heavy metals in water and tissues of *L. intestinalis* plerocercoids while there was significant negative (for Cu) correlation between the quantity the heavy metals in bottom sediment and tissues of *L. intestinalis* plerocercoids. This study supports the idea that cestodes aren't very useful to determine the heavy metal pollution in aquatic system when they are located in their intermediate host's body cavity.

Key words: *Ligula intestinalis*; tench; parasite; heavy metals; pollution; Turkey

Introduction

Many animal species are found either in some eutrophic conditions like habitats with an anthropogenic effect or sylvatic conditions with low exposure to xenobiotic substances but both conditions don't affect the animals' together (Sures *et al.*, 1997). Environmental conditions affect also aquatic hosts of parasites. Pollution may affect their health and cause extinction. These harmful changes are often associated with physiological reactions, which might be used as bioindicators (Segner, 1998; Segner & Braunbeck, 1998).

Some researchers indicated that cestodes, nematodes and acanthocephalans are able to accumulate considerable concentrations of heavy metals (Tenora *et al.*, 2000a; Baruš *et al.*, 2001; 2007; Turčeková *et al.*, 2002; Sures & Siddall, 2003; Thielen *et al.*, 2004; Tekin-Özan & Kır, 2005; 2007; Retief *et al.*, 2006; Genç *et al.*, 2008). For example, mean concentrations of lead and cadmium in *Monobothrium wageneri* Nybelin, 1922 obtained from intestine of tench (*Tinca tinca*) were 75 and 40 times higher than in the muscle of host (Sures *et al.*, 1997). Tenora *et al.* (2000a), investigated Cr, Pb and Cd concentrations in the *Ligula intestinalis* L., 1758 plerocercoids and three of its intermediate hosts and they found that the Pb, Cr and Cd levels in *L. intestinalis* plerocercoids were 15, 6 and 2.6 times higher than those in fish muscle. Tekin-Özan and Kır (2005) reported that concentrations of Cu, Fe, Zn and Mn in *Ligula intestinalis* plerocercoids in the body cavity of tench (*Tinca tinca)* from Kovada Lake in Turkey were respectively 1.6 – 37.4 times higher than in the muscle, liver and gill of the host and 2.2 – 691 times higher than in the water. Cestodes therefore show a high capacity of bioaccumulation of heavy metals located in the intestine of their respective final host. This idea was supported by Baruš *et al.* (2000) who investigated Cd and Pb concentrations in *L. intestinalis* and its host *Phalacrocorax carbo* and found that Cd and Pb levels in parasite were higher than the host's tissues.

Ligula intestinalis (L., 1758) is a pseudophyllidean cestoda and a widespread and important pathogen of fish (Dubinina, 1980; Aydoğdu & Öztürk, 2003). It has three hosts in its life cycle, the first intermediate host is a copepod, the second intermediate host is generally a cyprinid fish and the final host is a fish-eating bird (Dubinina, 1980; Ergönül & Altındağ, 2005).

The aim of this study was to determine concentrations of 4 different metals in *L. intestinalis* plerocercoids and its fish host (*Tinca tinca)* from Lake Beyşehir, Turkey. Furthermore, element levels in *Ligula intestinalis* plerocercoids were also compared with sediment and water of Beyşehir

Lake. The second aim was to indicate whether cestodes are more useful for accumulation bioindication of metals.

Material and Methods:

Description of study area
Beyşehir Lake (Fig. 1) is the largest freshwater lake in Turkey. The lake is located approximately 75 km from the city of Konya (37º 45´ N, 31º 30´ E). It is used for irrigation and electrical energy production. The lake is approximately 50 km long, 15 – 20 km wide and 10 m deep. Its area and volume are about 65.000 ha and 5 x 10^8 m^3, respectively (Tekin-Özan & Kır, 2006).

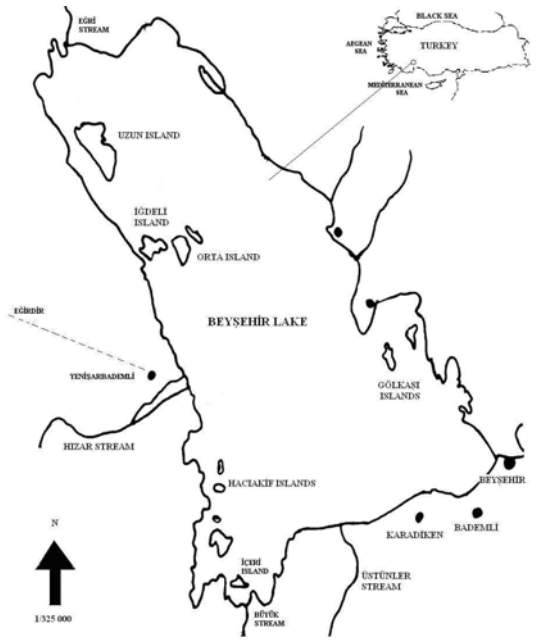

Fig. 1. Map of Beyşehir Lake (Turkey)

Sampling
A total of 40 tench (*Tinca tinca* L., 1758) were captured by fisherman in Beyşehir Lake. The sediments and water samples were also taken from Beyşehir Lake. The fish body weights were between 142 – 336 g and lengths were 21.5 – 29.6 cm.
The samples were brought to the laboratory on the same day. The fish were killed by a blow on the head. The cestodes were collected separately from each body cavity of different fish. Approximately 4 g of the muscle on the surface of the fish, the entire liver and two gill racers from each fish were dissected, washed with distilled water, dried with filter paper, weighed, packed in polyethylene bags and kept at -30 °C until analysis.

Analytical procedures
Water samples were analyzed directly. The wet samples (tissues, sediment and cestoda) that were weighed (1 g) before, 5 ml nitric acid (65 %) and 1 ml hydrogen peroxide were placed into the digestion bombs and digested in a microwave digestion system. After digestion, the samples were cooled to room temperature. The resulting solutions were made up to exactly 25 ml with high-quality deionized water and analyzed for Cu, Fe, Zn and Mn using a Perkin Elmer Atomic Absorpsion Spectrometer Analyst 800. The detection limit was 0.028 ppm for all elements.

Data analysis:
Element concentrations in the tissues of tench, its parasites and sediment were determined as $mg.kg^{-1}$ (wet weight) while water levels were determined as $mg.l^{-1}$. Pearson correlation coefficient was used to test for significant associations between the data obtained from tench tissues, its parasites, water and sediment. Significant was accepted with $P \leq 0.05$.

Results

The concentrations of heavy metals in fish tissues (liver, muscle and gill), *L. intestinalis* plerocercoids, water and sediment are summarized in Table 1. Cu, Fe, Zn and Mn were detected in water, sediment, *L. intestinalis* plerocercoids and different tissues of fish samples, while Cu was below detection limit (< 0.028 ppm) in muscle of tench and Mn in liver and muscle.
All elements analyzed were present at highest concentrations in liver, followed by gill and muscle (< 0.01). Only Zn was found to be significantly higher in the cestoda than in the liver and muscle, two (Cu and Zn) higher than in the gill (Table 1).
Mean concentrations of copper in *L. intestinalis* was 1.69 times higher than that of the gill. The zinc level in *L. intestinalis* was 2.13 times higher than that of the liver, was 5.53 times higher than that of the muscle and 3.33 times higher than that in the gill. Fe levels in the parasite were below the levels in the liver ($P < 0.01$), muscle and gill, but there were no significant differences ($P > 0.05$) between the parasite and muscle and gill. Mn was also found to be higher amounts in the gill than in the parasite. The concentrations of Mn were also not significant (> 0.05) different between the parasite and the fish tissues.
Mean concentrations of Cu, Fe, Zn and Mn in *L. intestinalis* were 55.3, 6.38, 279 and 14.2 times higher than in the water (Table 1). Fe was recorded in the highest amount in water. Mn concentrations in *L. intestinalis* and gill of fish and Zn concentrations liver of fish different significantly from the water ($P < 0.05$) (Table 2). No significant association was found for Cu and Fe concentrations of water and the metal levels of host tissues and *L. intestinalis* ($P > 0.05$) (Table 2).
Fe was recorded in the highest amount in bottom sediments. Mean concentrations of Zn in *L. intestinalis* was 1.12 times higher than in the bottom sediments (Table 1). Positive (for Fe and Zn) and negative (Cu and Mn) correlations were detected between the content in *L. intestinalis* and their quantity in bottom sediments of their reservoir (Table 3). The Cu concentrations in *L. intestinalis* and Fe

Table 1. Heavy metal concentrations in the liver, muscle, gill of fish (*Tinca tinca* L., 1758), *Ligula intestinalis* plerocercoids, water and bottom sediments

Elements	**Cu**	**Fe**	**Zn**	**Mn**
L. intestinalis				
Range	1.19-9.57	1.25-9.07	23.47-68.79	1.25-3.08
Mean (mg/kg ww)	5.53	4.34	44.69	2.14
St. Dv.	2.60	2.42	11.92	0.80
Liver of fish				
Range	8.86-28.53	55.97-393.45	13.46-26.82	BDL
Mean (mg/kg ww)	15.73	162.42	20.91	
St. Dv.	6.29	97.6	4.38	
Muscle of fish				
Range	BDL	3.26-9.49	4.90-15.47	BDL
Mean (mg/kg ww)		6.82	8.07	
St. Dv.		2.21	2.97	
Gill of fish				
Range	2.39-4.57	21.05-96.13	10.40-19.09	1.82-4.99
Mean (mg/kg ww)	3.26	50.62	13.4	3.74
St. Dv.	0.97	20.7	2.61	1.09
Water				
Range	0.08-0.15	0.10-2.74	0.02-0.42	0.02-0.52
Mean (mg/kg ww)	0.10	0.68	0.16	0.15
St. Dv.	0.01	0.94	0.18	0.24
Sediment				
Range	5.44-10.47	3466-15136	10.31-58.05	57.65-1029.22
Mean (mg/kg ww)	7.16	10397	39.82	484.19
St. Dv.	2.33	5263.03	20.66	415.18

ww, Wet weight; St.Dv., Standart deviation; BDL, Below Detection Limit

Table 2. Pearson correlation coefficient (r) and levels of significance determined for the relationship between the content of heavy metals in water, organs of fish and parasites

x – y	Metals	r	P	x – y	Metals	r	P
Water-*L.intestinalis*	Cu	0.964	> 0.05	Water-muscle of fish	Cu	-	-
(n = 24)	Fe	-0.086	> 0.05	(n = 24)	Fe	0.334	> 0.05
	Zn	0.051	> 0.05		Zn	0.011	> 0.05
	Mn	-0.774	< 0.05		Mn	-	-
Water-liver of fish	Cu	0.412	> 0.05	Water-gill of fish	Cu	0.740	> 0.05
(n = 24)	Fe	0.600	> 0.05	(n = 24)	Fe	-0.340	> 0.05
	Zn	0.758	< 0.05		Zn	0.497	> 0.05
	Mn	-	-		Mn	-0.836	< 0.05

Table 3. Pearson correlation coefficient (r) and levels of significance determined for the relationship between the content of heavy metals in bottom sediments, organs of fish and parasites

x – y	Metals	r	P	x – y	Metals	r	P
Sediment-*L.intestinalis*	Cu	-0.635	< 0.05	Sediment-muscle of fish	Cu	-	-
(n = 24)	Fe	0.653	> 0.05	(n = 24)	Fe	0.751	< 0.05
	Zn	0.167	> 0.05		Zn	-0.017	> 0.05
	Mn	-0.015	> 0.05		Mn	-	-
Sediment-liver of fish	Cu	0.138	> 0.05	Sediment-gill of fish	Cu	-0.035	> 0.05
(n = 24)	Fe	-0.477	> 0.05	(n = 24)	Fe	0.703	< 0.05
	Zn	-0.440	> 0.05		Zn	-0.372	> 0.05
	Mn	-	-		Mn	-0.393	> 0.05

concentrations in muscle and gill differed from the sediment ($P < 0.05$) (Table 3).

Discussion

In this study, some heavy metal levels in water, sediment, *Ligula intestinalis* plerocercoids and its host tissues from Beyşehir Lake were measured.

All of the analyzed metals were determined in water and sediment. The highest metal in water and sediment was Fe. Cu, Fe, Zn and Mn levels in *Ligula intestinalis* plerocercoids were 55.3, 6.38, 279 and 14.2 times higher than the water. Compared to sediment, only Zn levels in *Ligula intestinalis* plerocercoids was 1.12 times higher than that in the sediment. Similar results were given by Tekin-Özan and Kır (2005) and Turčeková *et al.* (2002).

There are various investigations about accumulations of heavy metals in tapeworm's plerocercoids paraziting the body cavity of the fish intermediate host and birds as final hosts. Tekin-Özan and Kır (2005) found that the Fe level in *Ligula intestinalis* plerocercoid 37.4, 2.4 and 5.6 times higher than the muscle, liver and gill. Tenora *et al*. (2000a) investigated Pb, Cr and Cd levels in *Ligula intestinalis* plerocercoids and found that Pb, Cr and Cd concentrations in *L. intestinalis* plerocercoids are 15, 6 and 2.6 times higher than in the fish muscle. Similarly, Sures *et al.* (1997) reported that Pb and Cd levels in *Monobothrium wageneri* was 75 and 401 times higher than the muscle of its host. In contrast to these studies, Svobodová et al. (1996) found that Hg levels in *Ligula intestinalis* plerocercoids were lower than in muscles of roach. In this study, only Zn levels were higher than liver (2.13 times) and muscle (5.53 times). Cu and Zn levels were 1.69 and 3.33 times higher than gill, respectively. Conversely to *Ligula intestinalis,* acanthocephalans and nematodes accumulate heavy metals in high concentrations. The mean concentrations of Fe and Zn in *Raphidascaris acus* (Bloch, 1779) were 68.4 and 86.9 times higher than in the muscle of the host (*Esox lucius*) (Tekin-Özan & Kır, 2007). Tenora *et al.* (2000a) reported that Pb, Cd and Cr concentrations in *Philometra ovata* were 106.11, 119.09 and 43.52 times higher than host. Lead and cadmium levels in *Pomphorhynchus laevis* Müller, 1776 were 2700 and 400 times higher than in the muscle of the host (Sures *et al.*, 1994a; Sures & Taracshewski, 1995). Similar results were also reported for other fish species infected with acanthocephalans and nematodes (Sures *et al.*, 1994b; Sures *et al.*, 1997; Genç *et al.*, 2008). Some researchers have investigated some heavy metal levels in *Confluaria capillarioides, Mesocestoides perlatus* and *Ligula intestinalis* and their final hosts (*Podiceps cristatus, Accipiter gentilis* and *Phalacrocorax carbo*) (Kráčmar *et al.*, 2000; Tenora *et al.*, 2000b; Baruš *et al.*, 2000) and found that Pb and Cd levels in cestodes were higher than in hosts liver and muscle tissues. Sures *et al.* (1997) indicated that cestodes tend to accumulate high metals when they are located in the intestine of their final host. In the present study, some heavy metals levels in *Ligula intestinalis* plerocercoids were determined lower than the host tissues. This may be due to their larval stages and location in the body cavity of the intermediate host. Feeding strategy and age of the *Ligula intestinalis* plerocercoids affect the heavy metal accumulation in cestodes.

The results presented here demonstrate that cestodes obtained from fish hosts accumulate some heavy metals higher rates than the water but lower than some fish tissues and sediment. This study supports the idea that cestodes aren't very useful to determine the heavy metal pollution in aquatic system when they are located in their intermediate host's body cavity.

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