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Original Research

Concentrations of Some Heavy Metals in Tench (*Tinca tinca* L., 1758), Its Endoparasite (*Ligula intestinalis* L., 1758), Sediment and Water in Beyşehir Lake, Turkey

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Abstract

Concentrations of Al, B, Ba, Cd, Cr, Ni, Pb and Sr were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) in the *Ligula intestinalis* plerocercoid (L., 1758), its host tissues (*Tinca tinca* L., 1758), sediment and water from Beyşehir Lake. Al, Ba, Cd, Cr, Ni, Pb and Sr were highest in sediment, while B was the highest in liver. Al in *Ligula intestinalis* plerocercoid was 6.91 times higher than in fish muscle. The Al, Ba and Sr levels in *Ligula intestinalis* plerocercoid, were 2.99x, 1.23x and 2.26x respectively, higher than those in fish liver. Compared with water, all heavy metal concentrations in *Ligula intestinalis* plerocercoid were higher. This study supports the idea that cestodes aren't useful to determine the heavy metal pollution in aquatic systems when they are located in their intermediate host's body cavity.

Keywords: *Ligula intestinalis*, heavy metal, pollution, fish, Turkey

Introduction

The difference between ecological or pollution indicators, and the presence or unexpected absence of the habitat or environmental quality expresses us bioaccumulation indicators. The organisms accumulate the polluted substances from their bodies, so that when tissues are analyzed an indirect forecast of prevailing environmental concentrations of these substances may be made [17].

Polluted bottom sediments represent an important and long permanent source of the contamination for an ecosystem. Pollutants' exchange into food chains and their subsequent accumulation depends on the ecology and food habits of water organisms. With respect to this fact, special attention has recently been given to the top of food pyramids [55].

Environmental conditions also influence the aquatic hosts of parasites. Pollution may affect their health and even cause extinction. These harmful changes are often associated with physiological reactions, which might be used as bioindicators [31]. Cestoda, nemotoda and acanthocephalans parasiting fish have been frequent subjects of investigations of heavy metals [6-8, 15, 29, 33-35, 40, 44, 47-53].

Mean concentrations of copper, iron, zinc and manganese in *Ligula intestinalis* plerocercoids from the body cavity of tench (*Tinca tinca*) were 1.6 and 37.4 times higher than in the muscle, liver and gill of the host and 2.2 and 691 times higher than the concentrations in the water [44]. Additionally, Tenora et al. [48] investigated Cr, Pb and Cd concentrations in the *L. intestinalis* plerocercoids and 3 of its intermediate hosts.

The present study was carried out in Beyşehir Lake, located in southwest Turkey (37º 45´ N, 31º 30´ E). The lake

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is approximately 50 km long, 15-20 km wide and 10 m deep. Its area and volume are about 65,000 ha and $5x10^8$ m^3, respectively [45]. It is an important visiting site for some bird species and falls in class "A" [56]. It is one of the national parks in Turkey [20]. It is fed by streams from Sultan and Anamas Mountains and groundwater. There are a lot of villages west of the lake, 15 fish processing systems around it and a weapons factory east of the lake. Agricultural production in the region is dominated by apple, grain and legumes. There are 33 islands in the lake [10]. The main sources of pollution come from the production of some foods and the wastewater of these industries.

Küçüködük [23] investigated the flora of Beyşehir Lake and found 340 species belonging to 74 familia and 222 genus. The dominant plants are: *Ceratophylletum demersi, Nymphaetum albae, Myriophylletum verticillatii, Phragmitetum communis, Typhetum angustifolia* and *Scirpietum lacustris*.

Hitherto, 38 different parasitic species have been determined from tench around the world [4, 21, 26, 28, 30, 43, 46, 54, 57]. Some of fish species have been reported as intermediate host of *L. intestinalis* plerocercoids, including cyprinids, catodtomids and percides [42]. Definitive hosts may be birds that eat infected fish [32].

Tekin-Özan et al. [46] reported that 52.99% of the investigated tench were infected with *L. intestinalis* plerocercoids. Ergönül and Altındağ [14] showed that, based-on age-weight, age-length and growth, *L. intestinalis* plerocercoids have an effect on the growth features of its second intermediate host, tench, in Lake Mogan. The examples given above show the importance of parasites in aquatic ecosystems.

Thus, the aim of this study was to determine if *L. intestinalis* plerocercoids is a useful bioindicator of heavy metal pollution in aquatic systems. Due to the tectonic construction of the lake, weapons factory, villages and fields Al, B, Ba, Cd, Cr, Ni, Pb and Sr were chosen to determine heavy metal pollution. Metal accumulation in *L. intestinalis* plerocercoid was compared to that in different tissues (muscle, liver and gill) of tench (*Tinca tinca*), sediment and water.

Material and Methods

Sampling and Sample Preparation

During the period of March to May 2005, 16 tench were caught by fisherman in Beyşehir Lake. The sediments and water samples were also taken from Beyşehir Lake. The fish body weights were between 210-245 g and lengths were 21.6-27.8 cm.

The samples were brought to the laboratory on the same day. The fish were killed by a blow to the head. The cestodes were collected separately from each body cavity of different fish. Approximately 4 g of the exapial 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. Samples were digested in a microwave digestion system by heating at 170ºC and 100 bar for 20 minutes. After digestion, the samples were cooled to room temperature. The resulting solutions were made up to exactly 25 ml with high-quality deionized water.

The metal analysis of samples (Al, B, Ba, Cd, Cr, Ni, Pb and Sr) were carried out using a Perkin Elmer Inductively coupled plasma optical emission spectroscope 5300 DV (ICP-OES). The concentrations of heavy metals are expressed as mg/kg wet weight of tissue. The absorption wavelength were 396.15 nm for Al; 249.77 nm for B; 455.40 nm for Ba; 228.80 nm for Cd; 267.71 nm for Cr; 231.60 nm for Ni; 220.35 nm for Pb and 407.77 nm for Sr.

The analytical procedure was checked using standard reference material dogfish muscle (DORM2) (National research council, Canada). Analytical blanks were prepared to determine the detection limits.

Data Analysis

Element concentrations in the tissues of tench, its parasites and sediment of the lake were determined as mg kg^{-1} (wet weight) while water levels were determined as mg l^{-1}. One-way analysis of variance (ANOVA) and Duncan's test ($p=0.05$) were used to access whether heavy metal concentrations varied significantly between materials, possibilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 12.0 for windows.

Results

The detection limit (3xSD of mean blank) for each element, the accuracy, the concentrations of the metals determined from the standard reference material and the certified values for the elements of DORM2 are given in Table 1. The accuracy for 8 of the elements ranged between 89 and 109%. The lowest accuracy was found for Cr with 89% and the highest Cd with 109%.

The concentrations of heavy metals (Al, B, Ba, Cd, Cr, Ni, Pb and Sr) in fish tissues (muscle, liver and gill), *Ligula intestinalis* plerocercoids, water and sediment are given in Table 2. As shown in Table 2, Al, Ba, Cd, Cr, Ni, Pb and Sr were detected in all materials. The B was undetectable in water.

Table 2 indicates that the sediment (142.29 mg/kg) had the highest aluminium concentrations accumulated, followed by gill. The Al level in *L. intestinalis* plerocercoid was 6.91 times higher than that of the muscle, was 2.99 times higher than that of the liver and 7.60 times higher than water. Aluminium concentrations in *Ligula intestinalis* plerocercoids did not differ significantly from those in the muscle,

Table 1. The summary of certified (mg/kg) and observed (mg/kg) value of reference material DORM2, accuracy and limits of detection (mg/l) of the ICP-OES analysis.

Element	DORM2 Certified values	DORM2 determined values	Accuracy (%)	Detection limit
Al	10.9±1.7	11.4±0.9	104	0.05
B	nc	-	-	0.007
Ba	nc	-	-	0.004
Cd	0.043±0.008	0.047±0.002	109	0.0012
Cr	34.7±5.5	31.2±1.2	89	0.0027
Ni	19.4±3.1	17.8±1.2	91	0.065
Pb	0.065±0.007	0.068±0.001	104	0.007
Sr	nc	-	-	0.005

nc - Element not certified in DORM2 (dogfish muscle certified reference material for heavy metal, National Research Council, Canada)

liver, gill and sediment (>0.05), but significant from water (<0.05). The highest boron concentration was measured in liver (5.75 mg/kg) followed by muscle and sediment and the lowest was in Ligula intestinalis plerocercoids. There is a significant difference (<0.001) between *Ligula intestinalis* plerocercoids and tissues of host. The sediment (20.42 mg/kg) had the highest barium concentrations accumulated, followed by the gill. The barium level in *L. intestinalis* plerocercoids was 1.23 times higher than liver. Between *L. intestinalis* plerocercoids and gill, and water the barium content differed significantly (<0.01). The highest cadmium concentrations were found in sediment (0.12) followed by liver and muscle. In the parasite, cadmium concentrations were 0.05 mg/kg and this value is lower than muscle and liver, equal with gill and 25 times higher than water (<0.05). The sediment (3.44 mg/kg) had the highest concentration of chromium followed by muscle (0.29 mg/kg). When the fish tissue means were compared it was observed that the cestodes had lower chromium concentrations. The chromium level in *L. intestinalis* plerocercoids was 30 times higher than water (<0.01). The sediment (4.05 mg/kg) had accumulated the highest concentration of nickel. When the fish tissue means were compared it was observed that the muscle had the highest accumulated nickel concentration, followed by the liver and gill. The Ni content of *L. intestinalis* plerocercoids was 40.8 times higher than water (<0.01). The highest concentration mean of lead accumulated was in sediment. The *L. intestinalis* plerocercoids concentration was 0.09 mg/kg and 15.78 times higher than water (<0.001). The strontium accumulation had the highest mean concentrations in the sediment (29.29 mg/kg) followed by gill. The *L. intestinalis* plerocercoids concentration (2.61 mg/kg) was 237.27 times higher than water (<0.01).

Discussion

In this study, the accumulation of Al, B, Ba, Cd, Cr, Ni, Pb and Sr in plerocercoids of *Ligula intestinalis*, its host tissues, sediment and water had been measured.

The sediment had the highest concentration in 7 (aluminium, barium, cadmium, chromium, nickel, lead and strontium) out of 8 elements. In aquatic systems, metals are transported either in solution or on the surface of suspended sediments [12]. Due to their strong affinity for particles [24], suspended matter or bottom sediment accumulate the metals [11]. The heavy metals may be in sediment through indirect discharge or from atmospheric deposition at the plant [13].

The water had the lowest concentrations in all elements. Karadede and Ünlü [19] reported that the lowest metal concentrations were found in water among studied material (water, sediment and fish). Similar results were indicated by different researchers from Hazar Lake [25], Uluabat Lake [5] and Habbaniya Lake [2]. The low concentration in water can be due to the fact that sediments, aquatic plants and organisms may accumulate heavy metals from the water column. The investigation done by Al-Saadi et al. [2] support this idea. They found the highest heavy metal concentrations in sediment and aquatic plants in Habbaniya Lake.

Fish are relatively long-lived, mobile and therefore good indicators of long-term effects and habitat changes [17]. They are top of the aquatic food chain and are eaten by man [17]. These organisms take up pesticides and metals from the aquatic ecosystem via food, drinking water and the gills. In the present study, the second highest metal concentrations were found in liver, while the lowest were detected in muscle tissues. Different investigators have reported similar results [18, 22, 45]. The accumulation of metals in the liver could be due to the tendency of the elements to react with the oxygen carboxylate, aminogroup, nitrogen and/or sulphur of the mercapto group in the metallothionein protein, whose concentration is highest in the liver [3]. Metal concentration in the gills could be due to the element complexing with the mucus, which is impossible to remove completely from between the lamellae before tissue is prepared for analysis. Thus different metals are accumulated here with high concentrations [16]. However, the muscle tended to accumulate less metal. Because the muscle is not an active tissue for heavy metal accumulation [18].

On heavy metal bioaccumulation and concentrations in tapeworms plerocercoids parasitizing the body cavity of the fish intermediate host, there are various data and opinions. Tenora et al., [48] found that Pb, Cr and Cd concentrations in *Ligula intestinalis* plerocercoids are 15, 6 and 2.6 times higher than in fish muscle. Similarly, Tekin-Özan and Kır [44] reported that the iron level in *Ligula intestinalis* plerocercoid is 37.4 times higher than that of the muscle, was 2.4 times higher than that of the liver and 5.6 times higher than that in the gill. Retief et al. [29] investigated heavy metal concentrations in *Bothriocephalus acheilognathi* from the largemouth yellowfish (*Labeobarbus kimberleyensis*).

Table 2. Heavy metal concentrations in Ligula intestinalis plerocercoids, different organs of Tinca tinca, sediment and water from Beyşehir Lake (Turkey).

Elements		M	L	G	Li	S	W
Al	Mean	1.77^{a}*	4.09^{a}	12.46^{a}	12.24^{a}	142.29^{a}	1.61^{b}
	SD	0.01	2.18	5.89	7.53	66.74	0.87
	Min	1.76	1.95	6.82	1.06	76.72	0.80
	Max	1.78	6.33	18.58	25.09	210.15	2.53
B	Mean	4.81^{b}	5.75^{b}	4.55^{b}	4.32^{a}	4.75^{a}	BDL**
	SD	0.70	0.12	0.18	0.36	1.38	
	Min	4.04	5.67	4.40	3.97	3.51	
	Max	5.41	5.90	4.75	4.70	6.24	
Ba	Mean	1.12^{a}	0.30^{a}	13.73^{b}	0.37^{a}	20.42^{a}	0.02^{b}
	SD	0.15	0.11	3.77	0.14	9.66	0.01
	Min	0.98	0.19	9.84	0.24	10.98	0.01
	Max	1.30	0.42	17.37	0.55	30.30	0.03
Cd	Mean	0.08^{cd}	0.10^{de}	0.05^{c}	0.05^{b}	0.12^{a}	0.002^{e}
	SD	0.01	0.01	0.005	0.02	0.02	0.0004
	Min	0.07	0.09	0.05	0.03	0.10	0.001
	Max	0.10	0.11	0.06	0.08	0.15	0.002
Cr	Mean	0.29^{a}	0.19^{a}	0.28^{a}	0.18^{a}	3.44^{a}	0.006^{b}
	SD	0.03	0.01	0.02	0.05	1.75	0.001
	Min	0.25	0.18	0.26	0.10	1.96	0.005
	Max	0.33	0.20	0.31	0.23	5.38	0.008
Ni	Mean	2.43^{bc}	0.66^{ab}	0.36^{a}	0.20^{a}	4.05^{a}	0.0049^{c}
	SD	2.11	0.35	0.17	0.19	1.92	0.002
	Min	0.29	0.30	0.19	0.06	2.16	0.002
	Max	4.52	1.00	0.54	0.49	6.00	0.007
Pb	Mean	0.20^{a}	0.35^{a}	0.13^{a}	0.09^{a}	1.50^{a}	0.0057^{b}
	SD	0.11	0.13	0.02	0.03	0.78	0.0009
	Min	0.10	0.22	0.11	0.05	0.73	0.004
	Max	0.33	0.49	0.15	0.12	2.29	0.006
Sr	Mean	3.10^{a}	1.15^{a}	12.79^{b}	2.61^{a}	29.29^{a}	0.0110^{c}
	SD	0.78	0.33	11.05	0.70	4.16	0.0007
	Min	2.38	0.80	1.64	1.90	25.28	0.0103
	Max	3.93	1.46	23.74	3.49	33.60	0.0118

* Data shown with different letters are statistically significant at the $P<0.05$ level.
**BDL-Below Detection Limit
M: Muscle of infected fish, L: liver of infected fish, G: gill of infected fish, Li: Ligula intestinalis plerocercoid, S: Sediment, W: Water

They found that 8 elements out of the 23 elements measured the cestodes have accumulated the highest metal concentrations, and in 7 elements out of 23 elements the cestodes had the second highest metal accumulation recorded. By contrast with these studies, Pascoe and Mathey [27] found that Cd concentrations in *Schistocephalus solidus* (Müller, 1776) plerocercoids are lower than those in the body of *Gasterosteus aculeatus* L. and Svobodová et al., [41] state that Hg concentrations in *Ligula intestinalis* plerocercoids were lower than those in muscles of roach. In this study, only the aluminium level was higher than muscle (6.91 times). Aluminium, barium, and strontium levels were 2.99, 1.23 and 2.26 times higher than liver, respectively. On the contrary, *L. intestinalis*, acanthocephalans accumulate the heavy metals in high concentrations. The mean concentrations of lead and cadmium in Pomphorhynchus laevis were 2700 and 400 times higher than in the muscle of the host and 11,000 and 27,000 times higher than in the water [36, 38]. Similar results were also reported in other fish species infected with acanthocephalans [37, 39]. Various researchers [9, 36] reported that the larval stages of acanthocephalans in their intermediate hosts tend to accumulate low metals like cestodes. Sures et al. [39] described cestodes showing a capacity to accumulate the heavy metals when they are located in the intestine of their final host. This comparatively low accumulation of heavy metals in *Ligula intestinalis* plerocercoids may be related to their larval stages and location in the body cavity of the host. Heavy metal accumulation in plerocercoids can also be affected within a fish species by a change of their feeding strategy and by differences in food components, as was found, for uninfected and infected roach by Adámak et al. [1].

Conclusions

The proposed method was efficient for simple, rapid and reliable assessment of some heavy metals in the water, sediment, fish tissues and cestoda. The accuracy of the method was checked and confirmed by standard reference materials. The recoveries of the heavy metals standard reference materials were in the range of 89-109%.

The results presented here demonstrate that cestodes from fish hosts accumulate some heavy metals higher rates than the water but lower than some fish tissues and sediment. This study support 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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