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Original paper

Corynosoma caspicum (Acanthocephala, Polymorphidae), as a heavy metal bioindicator in the fish *Gasterosteus aculeatus* from the Caspian Sea, northern Iran

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ABSTRACT. Previous marine biology studies found that the concentration of heavy metals in some parasites of fish such as acanthocephalans can be a proper bioindicator. Therefore, we attempted to measure five heavy metal concentrations in the tissues of the fish *Gasterosteus aculeatus* (*G. aculeatus*) and its acanthocephalan parasites, *Corynosoma caspicum* (*C. caspicum*) from the Southern Caspian Sea, northern Iran. *G. aculeatus* (three-spined stickleback) was collected from the south of the Caspian Sea, Mazandaran Province, northern Iran. After tissue preparation, the heavy metal concentrations in fishes and acanthocephalans were obtained using the tissue dissolution technique and an atomic absorption spectrophotometer. The concentrations of chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) in the skin, liver, muscle, and intestine tissues of the fish and its parasites, *C. caspicum*, were measured and compared. Eighty (32%) of 250 collected fish were infected by at least one acanthocephalan parasite. The Cr indicated the highest concentration (5.329±3.275) of the heavy metals in acanthocephalan, even more than the skin, liver, and muscle of infected fishes. Cd had the lowest concentration (0.0333±0.0075) of heavy metals in acanthocephalan, but it was still higher than the concentration in the infected fishes' skin, liver, muscle, and intestine tissues. Our findings indicated that *C. caspicum* parasites can be considered extremely sensitive early-alert bioindicators, particularly in sensitive and under-threat environments with low pollution levels.

Keywords: *Gasterosteus aculeatus*, *Corynosoma caspicum,* acanthocephalan, fish, heavy metal, bioindicator

Introduction

Heavy metal pollution in the aquatic ecosystem has attracted serious concern in recent years. The increase of industrialization and the discharge of wastes into the environment might be the main sources of pollution, especially in developing countries [1]. The pollution of the coastal and marine environments has become a serious threat to both marine ecosystems and humans who are marine-dependent as well [2]. Unlike in public opinion, parasites have some advantages for their hosts. Certain fish parasite species have been identified as being highly sensitive to aquatic

Table 1. The concentration of heavy metals including Cr, Cd, Pb, Zn and Cu in different organs of infected and non-infected *Gasterosteus aculeatus* from the southern Caspian Sea, northern Iran

Heavy metals	Fish status	Skin	Liver	Muscle	Intestine
	Infected fish				
Cr		3.081±2.285	3.398±1.228	10.413±3.845	4.345±5.248
Cd		0.0303±0.0113	0.013± 0.010	0.0253±0.0075	0.0326±0.0066
Pb		0.17±0.081	0.1346±0.0561	0.0753±0.0421	0.0306±0.026
Zn		2.374±1.068	0.4673±0.2537	1.272±0.408	0.3396±0.0730
Cu		0.028±0.017	0.2013±0.0770	0.016± 0.012	0.0113±0.0070
	Non-infected fish				
Cr		6.859±4.291	2.825±0.5178	5.649±1.128	3.389±1.294
Cd		0.996±1.705	0.0256±0.0090	0.0253±0.0146	0.0106±0.0047
Pb		0.17±0.095	0.10262±0.0652	0.2316±0.1032	0.0466±0.0345
Zn		4.020±3.52	0.3130±0.2268	2.637±1.129	1.175±1270
Cu		0.143±0.0401	0.4053±0.5223	0.0476±0.0174	0.051±0.011

(Column group header "Fish organs" spans Skin, Liver, Muscle, Intestine.)

pollutants, either in terms of their physiological response or their ability to accumulate specific toxins in a dose- and time-dependent manner [3]. Fishes are infected with three major groups of helminths: Cestoda, Nematoda and Acanthocephala. About 20,000 to 30,000 helminth species have been reported worldwide, which cause heavy losses to the fish industry [4]. Several studies have revealed that heavy metal concentrations in some helminth parasites are much higher than in their hosts [5–8]. Most studies have indicated that acanthocephalans and cestodes can be used as bioindicators to obtain the concentration of heavy metals [1,9]. The excellent characteristics of acanthocephalans in absorption of heavy metal pollutants were found to be related to their anatomy, metabolism, physiology, and localization in the host [10]. There are only a few studies about the bioabsorption of intestinal helminths in different hosts from Iran [7,9,11,12].

In the present study, the chromium (Cr) and cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) concentrations were measured in the tissues of the fish *Gasterosteus aculeatus* L., 1758 (*G. aculeatus*) and its acanthocephalan parasite, *Corynosoma caspicum* Golvan and Mokhayer, 1973 (*C. caspicum*) from the Southern Caspian Sea, Mazandaran Province, northern Iran.

Materials and Methods

Fish sampling

Between September 2012 and August 2014, 250 *G. aculeatus* were caught using gillnets from the southern coast of the Caspian Sea, Babolsar City, Mazandaran Province (53°6′5E, 36°23′3N), northern Iran. The fishes were transported to the Caspian Sea Ecology Research Center Laboratory. The genus and species of fish were identified by an expert using the key reference [13]. Then biometric features were measured for each fish. The fishes were frozen at –20°C using the parasitological protocol for acanthocephalan in the laboratory of the Department of Parasitology, Medical School, Mazandaran University of Medical Sciences, Sari, northern Iran. Prior to beginning of the experimental studies on the fishes, all the dissection instruments were washed and sterilized with 1% ammonium-EDTA (Merck, Germany). Dissection was performed in sterile conditions, the numbers of acanthocephala in each fish were counted, and the wet weight was recorded. Morphological and morphometric characteristics of each specimen were drawn using Camera Lucida, at 400× magnification, which the characteristics were confirmed based on key references [14,15]. In addition, the parasite specimens were sent to Iranian

Table 2. The concentration of heavy metals (Cr, Cd, Pb, Zn and Cu) in different organs of infected fishes in comparison to the *Corynosoma caspicum* tissue from the southern Caspian Sea, northern Iran

Heavy metals	Fish organs				Acanthocephalan parasite
	Skin	Liver	Muscle	Intestine	
Cr	3.081±2.285*	3.398±1.228*	10.413±3.845*	4.345±5.248*	5.329±3.275*
Cd	0.0303±0.0113*	0.013±0.010*	0.0253±0.0075*	0.0326±0.0066*	0.0333±0.0075*
Pb	0.17±0.081*	0.1346±0.0561*	0.0753±0.421*	0.0306±0.026*	0.143±0.93*
Zn	2.374±1.068**	0.4673±0.2537*	1.272±0.408**	0.3396±0.0730*	0.311±0.321*
Cu	0.028±0.017*	0.2013±0.0770**	0.016±0.012*	0.0113±0.0070*	0.038±0.0029*

* Not significant; ** Significant

Parasitology National Museum (IPNM) at Veterinary School of Tehran University for species confirmation. Also, skin, liver, muscle and intestine of the fishes were isolated, washed with double-distilled water and stored at –20°C until metal analysis assay.

Metal analysis

Samples had a rang weight of 0.0005 g to 2 g and were digested in the mixture of 5 ml nitric acid (65%, Merck, Germany), 5 ml sulfuric acid (75%, Merck, Germany), and 1 ml chloridric acid (Merck, Germany) for one hour. For rapid digestion, the specimens were heated up to 70°C for 2 hours. Finally, the resultant solution was diluted up to 20 ml with distilled water and analyzed for heavy metals [7,16]. The concentrations of standard solutions and heavy metals were analyzed using atomic absorption spectrometry. Statistical methods such as two-way analysis of variance (ANOVA) and the t-test were used to analyze the data.

Ethical statement

During all stages of the study, all applicable international, national, and/or institutional ethical guidelines for the care and use of animals were followed. The fishes were caught according to the ethical approval of the Caspian Sea Ecology Research Center.

Results

Parasite identification

Among the total of 250 collected fish, 80 (32%) were infected with at least one acanthocephalan worm. Length of an adult acanthocephalans was 4.3±2.7. Based on the morphological details and the IPNM report, all specimens were identified as *C. caspicum.* The skin, liver, muscle, and intestine of each fish were isolated and analyzed for heavy metals, including Cr, Pb, Zn, Cu, and Cd. The results were compared to the non-infected fish organs as well as parasite tissue.

A comparison of the heavy metals in different organs of infected and non-infected fishes

According to table 1, the concentration of the heavy metals including Cr, Zn, Cd, and Cu in the skin of infected fish was significantly lower than that of non-infected fishes (P<0.05). The concentration of Cu in the liver of non-infected fishes was higher than that of infected fish, whereas there was no significant difference. Moreover, the concentration of Zn in the muscle and intestine of non-infected fishes was significantly higher than infected fishes (P<0.05). Infected fishes had a non-significant higher concentration of Cr in their livers, muscles, and intestines compared to non-infected fishes. The concentration of Pb and Zn in liver and Cd in intestine indicated a higher concentration in infected than non-infected fishes, but the difference was not significant.

A comparison of the concentration of heavy metals in different organs of infected fishes and tissue of Corynosoma caspicum

The concentration of Cr in acanthocephalan tissue was higher than in skin, liver, and intestine. Cd had the least concentration of heavy metals in parasites but was higher than all the tissues of infected fishes. Pb showed a higher concentration in acanthocephalan than in liver, muscle, and intestine of infected fishes, and Cu was higher than in skin, muscle, and intestine of fishes. The concentration of Zn in skin and muscle and Cu in the liver of fishes was significantly more than parasite tissue ($P<0.05$) (Tab. 2).

Discussion

The present preliminary study, for the first time in Iran, revealed different concentrations of heavy metals including Cr, Cd, Pb, Zn, and Cu in different fish organs such as skin, liver, muscle, and intestine of both infected and non-infected fishes, *G. aculeatus* and *C. caspicum*, both of which are acanthocephalan parasites. The results showed that the concentration of most heavy metals was higher in infected than in non-infected fish, especially in the skin. The acanthocephalans had a higher concentration of Cd than all the tissues of their host. The concentration of Cr in acanthocephalan was greater than the skin, liver, and intestine of infected fishes. The concentration of Pb in acanthocephalan was higher than in the liver, muscle, and intestine of infected fishes. Also, Cu in acanthocephalan had a higher concentration than in the skin, muscle, and intestine of infected fish. Zn was the only heavy metal that had a lower concentration in parasites than in all fish organs. Therefore, acanthocephalan parasites may actually have a potential impact on the health of their hosts. The heavy metals forming organometallic complexes pass through the bile duct into the small intestine of fishes. Also, acanthocephalan parasites are not able to synthesize their own cholesterol and fatty acids, although they are necessary for bile salts. Hence, they have become well-organized in taking up heavy metals from bile salts of the host intestinal lumen and play a critical role as heavy metal filters or absorbents. Consequently, it will diminish the uptake quantity of heavy metals by the hosts' intestinal wall [1,17].

These results are in line with other studies and approve the heavy metal absorption ability of acanthocephalan in comparison with its hosts. In a study by Sures and Reimann [18] in the South Shetland Islands, most of the heavy metals were found in significantly higher concentrations in the acanthocephalan, *Aspersentis megarhynchus* than in the liver, muscle, and intestine of its host, *Notothenia coriiceps*. They reported Pb, Cd, Ag, Ni, and Cu as the main metals in respect of their bioconcentration in the acanthocephalan. In other studies, Sures and Siddall [19] described how *Pomphorhynchus laevis* (*P. laevis*) indicated higher concentrations of Pb than the intestine of its fish host, *Leuciscus cephalus* and *Paratenuisentis ambiguous* demonstrated higher concentrations of Cd and Pb than their hosts. In addition, Schludermann et al. [20] indicated higher concentrations of Cd, Pb, and Zn in the acanthocephalan worm *P. laevis* in comparison with the liver, muscle, and intestine of infected fishes. In a study from Hungary, in the Danube River, a negative correlation was reported between the number of acanthocephalans *P. laevis* and metal levels in tissues (liver, muscle, intestine, and kidney) of barbel (*Barbus barbus*). Also, the acanthocephalan indicated higher amounts of ten of twenty-one studied heavy metals than all barbel organs [8]. In another study from Slovakia, a negative correlation was found between heavy metal concentrations in perch (*Perca fluviatilis*) organs and the parasites *Acanthocephalus lucii* and a tapeworm, *Proteocephalus percae* [21]. Abdel-Mawla et al. [22] in the Suez Canal area reported a lower average residue of heavy metals (Cd, Pb, Zn, Cu) in fish organs infected with acanthocephalan parasites compared to non-infected fish. Also, they stated that acanthocephalan parasites can accumulate heavy metals more than the tissues of their fish hosts. Lacerda et al. [23] stated that the abundance of acanthocephalan may be negatively influenced by organic pollution in the marine environment, supporting the potential use of fish parasites as bioindicators of marine areas. There are a few studies about the bioindication of parasitic helminths in Iran. Firstly, Malek et al. [7] conducted a study in the Persian Gulf, southern Iran, and reported several times higher concentrations of Pb and Cd in two cestoda species, named *Anthobothrium* sp., and *Paraorigmatobothrium* sp., compared to their shark host (*Carcharhinus dussumieri*). Teimoori et al. [12] investigated the heavy metal bioabsorption capacity of intestinal worms in urban rats (*Rattus rattus* and *Rattus norvegicus*) of Tehran, the capital of Iran. They found significantly more Cr and Cd in the liver, kidney, and muscle of uninfected rats than in infected rats with *Moniliformis moniliformis* and cestoda, including *Hymenolepis diminuta* and the larval stage of *Taenia taenaeiformis* (*Cysticercus fasciolaris*). Also, they found a higher concentration of these metals in parasites compared to host tissues. Najm et al. [9] reviewed the role of helminths as heavy metal bioindicators in aquatic ecosystems and reported that helminthic parasites, especially acanthocephalan and cestoda, could be employed as bioindicators to find the heavy metal concentration. Overall, according to our findings, the acanthocephalans are beneficial in terms of heavy metal absorption and indication.

Our results indicated that acanthocephalan parasite (*C. caspicum*) is prevalent in the fish host *G. aculeatus*. Also, it was concluded that Cr ion and skin were the highest concentrated heavy metal pollutant and the most infected fish organ in *G. aculeatus*. Our results confirmed that acanthocephalan worms are sensitive bioindicators for heavy metal pollution and can role as heavy metal filters for their fish host.

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