Science of the Total Environment 575 (2017) 836–840

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Contracaecum sp. parasitizing *Acestrorhynchus lacustris* as a bioindicator for metal pollution in the Batalha River, southeast Brazil

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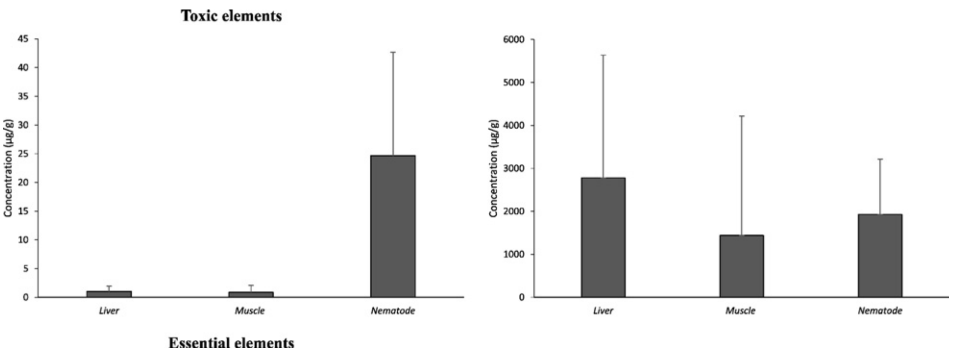
HIGHLIGHTS

- The potential of larvae *Contracaecum* sp. as indicators of contamination by metals using ICP-MS was evaluated.
- 12 of the 13 analyzed elements were detected in higher concentrations in parasites than in host tissues.
- In the parasites, the concentration of toxic elements was approximately 40 µg/g and the essential elements were 1900 µg/g.
- The highest BCF for the concentration of metals in parasites and muscle were Cu and Ni while in the liver was Cd.
- *Contracaecum* sp. show good accumulation capacity for the elements considered essential and those considered to be toxic.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form 15 September 2016
Accepted 16 September 2016
Available online 25 September 2016

Editor: D. Barcelo

Keywords:
Heavy metals
Inductively coupled plasma mass spectrometry
Larvae nematode
Biomonitoring
Tietê-Batalha River basin

ABSTRACT

Pollution in aquatic ecosystems due to negative human activities remains a problem in both freshwater and marine environments and is an ongoing subject of research. Several studies have shown that some fish parasites can be used as a tool for biomonitoring because they demonstrate higher metal accumulation capacity compared to their host tissues. However, compared to acanthocephalans, information regarding the absorption mechanisms and accumulation rates in nematodes is relatively limited. Here, we evaluated the potential of larvae *Contracaecum* sp. (L3) as indicators of contamination by metals by analyzing thirteen element concentrations: magnesium (Mg), aluminum (Al), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), barium (Ba), and lead (Pb) in the parasites and host *Acestrorhynchus lacustris*, using inductively coupled plasma mass spectrometry. Twelve of the thirteen analyzed elements were detected in at least 2-fold higher concentrations (e.g. Ni) and were up to approximately 50-fold higher (e.g. Pb) in parasites than in host tissues, including elements known for their high toxicity (As, Cd, Pb) and those considered to be essential (Cu and Zn). Our results suggest that *Contracaecum* sp. larvae can be used as bioindicators of metal contamination because even in early stages of development, numerous essential and non-essential elements were accumulated, making this system a useful tool for monitoring polluted environments.

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http://dx.doi.org/10.1016/j.scitotenv.2016.09.132
0048-9697/

1. Introduction

Pollution in aquatic ecosystems due to negative human activities remains a problem in both freshwater and marine environments, as pollutants are continuously poured into these environments, raising concerns about the impacts on these ecosystems (Khan and Thulin, 1991) and making it an important subject of many discussions. In order to determine the biological levels of pollutants in the environment, bioindicators are extremely useful tools in addition to the chemical analyses of water and sediment, which primarily describe the total concentrations of the respective pollutants (Thielen et al., 2004).

In addition to currently used indicators, certain parasites have been widely used as bioindicators of accumulation, as environmental pollution significantly affects these organisms (Vidal-Martínez et al., 2009; Vidal-Martínez and Wunderlich, 2016), altering their health and prevalence, distribution, and pathogenicity (Khan and Thulin, 1991; Thielen et al., 2004). Associations between pollutants and parasites can have positive or negative effects depending on the parasite taxa and pollutant type. In most cases, the effects of metals are negative, in which parasitism decreases with increasing exposure to pollutants, particularly in freshwater environments (Blanar et al., 2009). In addition to pollutants, parasites can affect the health of organisms, and the responses of some of these organisms to pollutants and parasites are very similar (Lafferty, 2008; Lafferty and Kuris, 2005; Sures, 2008).

Palm (2011), Sures (2008), and Sures et al. (1999a) described several advantages of using parasites as indicators of metal contamination and/or accumulation. For example, there is a wide variety of host-parasite combinations, host and parasite collection is easy, and some species show a high metal tolerance. In contrast, disadvantages include the short life cycle of parasites compared to their hosts and a lack of knowledge regarding the metal absorption rate and route and capture mechanisms of metals by parasites.

Despite the preference of using acanthocephalans as sentinels because of their high accumulation capacity, nematode larvae may also show a good ability to accumulate metals, particularly those considered as essential, suggesting that this species can be used as a pollution indicator (Baruš et al., 2007; Morsy et al., 2012; Nachev et al., 2013; Nagy, 1999). However, little is known about the accumulation rates in nematode larvae parasites of fish because of their larval stage variability and their frequency of encapsulation in host tissues, which may influence nutrient and pollutant uptake (Nachev et al., 2013).

Species in the genus *Contracaecum* are very important in public health because of their high zoonotic potential (Vidal-Martínez et al., 1994). Adults in this genus are parasites of the digestive tracts of ichthyophagous birds and marine mammals, while the larvae are commonly found in internal organs (peritoneal cavity, mesentery, stomach, intestine, and liver) of fish, which act as intermediate or paratenic hosts (Moravec, 1998). The zoonotic potential of *Contracaecum* in humans is unclear because of the limited number of reported cases (Benigno et al., 2012; Buchmann and Mehrdana, 2016), but experimental studies of terrestrial mammals have confirmed the susceptibility to infection and highlight the zoonotic importance of the genus (Barros et al., 2007; Vidal-Martínez et al., 1994).

In this study, we evaluated the potential of the larvae, *Contracaecum* sp., as indicators of metal contamination by comparing the concentrations of 13 elements detected in parasites and different host tissues using inductively coupled plasma mass spectrometry (ICP-MS).

2. Material and methods

2.1. Study area

The Batalha River water catchment lagoon is located between the limits of the cities of Bauru (22°18′54″S and 49°03′39″W) and Piratininga (22°24′46″S and 49°08′04″W) in the southeastern state of São Paulo. With an area of approximately 170.000 m^2 and water volume of 1.256.040 m^3/month, the lagoon's water supplies approximately 45% of the urban population of the city of Bauru. The agricultural and industrial activities in the mechanical and food areas, predominantly sugar and alcohol (Comitê da Bacia Hidrográfica do Tietê-Batalha, 2016), have triggered changes in water quality and exposed headwater areas to increasing erosion of land adjacent to the river; this has led to the siltation of its margins and pollution of its waters (Silva et al., 2009). Furthermore, most of the river near cities has no sewage treatment or treats only a percentage of the water, while remaining waste enters the river *in natura*. Even in extremely impacted areas, water in the Batalha River is considered to be of sufficient quality for consumption according to the official regulatory agency (Santos and Heubel, 2008).

2.2. Sampling and processing of hosts and parasites

A total of 32 specimens of *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes: Acestrorhynchidae) were collected between May and September 2013 from the Batalha River water catchment lagoon. In the laboratory, the fishes were necropsied and their organs were analyzed separately under a stereomicroscope to collect parasites. The *Contracaecum* sp. larvae (Nematoda: Anisakidae) were collected from the stomach, intestine, liver, gonads, swim bladder, and heart. From fish, muscle and liver samples were also taken which, along with the parasites, were frozen separately at −20 °C until further processing for metal analysis.

2.3. Metal analysis

After thawing, 150 mg of each tissue (liver and muscle) and parasites were digested using inorganic reagents (FLUKA, P.A., São Paulo, Brazil) (HNO_3 and $3H_2O_2$) in an alternate accessory and digester with a microwave oven (DGT 100 plis, Provecto Analítica, São Paulo, Brazil). The program used was as follows: 1st step: 2 min at 200 W, 2nd step: 8 min at 400 W, and 3rd step: 10 min at 600 W. Concentrations of the elements magnesium (Mg), aluminum (Al), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), barium (Ba), and lead (Pb) were determined using an inductively coupled plasma mass spectrometer (ICP-MS, Elan 6100, Perkin Elmer, Waltham, MA, USA). To identify the analytes, an analytical program was created; because no certified reference material is available, we used a similar matrix developed by the Instituto de Pesquisas Energéticas e Nucleares (Moreira et al., 2007; Moreira et al., 2013).

2.4. Statistical analysis

In order to express the parasite accumulation capacity for the different organs, the ratio $C_{[parasite]}/C_{[organ]}$ (bioconcentration factor; BCF) was calculated according to Sures et al. (1999a). After conducting the Shapiro–Wilk test of normality, one-way ANOVA was used to test for significant differences ($p \leq 0.01$) between the element concentrations in the analyzed tissues and parasites.

3. Results

3.1. Analytical process

The accuracy of the reference material, as well as the detection limits of each element, are listed in Table 1. The accuracy varied between 88% and 109%, which is considered a reliable analysis. The detection limits for essential elements (e.g. Mg, Fe, and Zn) as well as the quantification limits were higher than those considered non-essential because of their higher natural occurrence.

Table 1
Metal concentrations in the certified reference material (CRM), precision, and detection limits determined by ICP-MS analysis.

Elements	CRM values ± SD (mg/kg)	Accuracy (%)	Detection limit (μg/g)	Quantification limit (μg/g)
Mg	0.351 ± 0.014	88	0.06	0.21
Al	n.c.	–	0.01	0.01
Ti	n.c.	–	0.02	0.02
Cr	0.90 ± 0.06	109	0.03	0.09
Mn	21.2 ± 0.9	103	0.02	0.06
Fe	373 ± 6	111	0.14	0.48
Ni	n.c.	–	0.05	0.05
Cu	n.c.	–	0.02	0.02
Zn	103 ± 1	96	0.16	0.52
As	15.8 ± 0.5	92	0.01	0.03
Cd	0.654 ± 0.013	97	0.05	0.17
Ba	n.c.	–	0.01	0.03
Pb	n.c.	–	0.01	0.03

3.2. Element concentrations in A. lacustris and Contracaecum sp.

The 32 specimens of *A. lacustris* analyzed had a mean length and weight of 16.27 ± 3.37 cm and 67.45 ± 58.0 g, respectively. The *Contracaecum* sp. larvae had a prevalence of 97% and mean intensity of 37.52 ± 35.73, and the intestine, stomach, and liver showed the highest infection rates. Comparison of the mean concentrations of elements in the two analyzed host tissues revealed that the liver contained significantly higher concentrations (ANOVA, $p \le 0.05$) in three (Fe, Ni, and Zn) of 13 elements, while the muscle showed higher concentrations for only Cr and Cd (Table 2).

A comparison of the element concentrations in the larvae of *Contracaecum* sp. and the analyzed tissues of *A. lacustris* demonstrated that parasites contained significantly higher concentrations (ANOVA, $p \le 0.05$) of 12 of the 13 elements analyzed (Table 2). Mg was highest in both tissues (liver and muscle), whereas Mn and Fe were highest in the liver.

The concentrations of toxic elements (e.g. Al, Ti, and Cr) in both organs were below 1.40 μg/g, whereas the elements considered to be essential (e.g. Mg, Fe, and Zn) were present at > 2000 μg/g. In the parasites, the concentration of toxic elements was approximately 40 μg/g and the essential elements were 1900 μg/g (Fig. 1 and Table 3). BCFs enable comparison of the helminth relative accumulation capacity in various analyzed host tissues. The BCFs of the elements analyzed in *Contracaecum* sp. compared to those in the host liver (Table 4) were as follows (in decreasing order): Cd > Al > Pb > Ba > As > Cr > Ti > Cu > Ni > Zn > Mn > Fe > Mg. In contrast, the order for BCFs in parasites and host muscle were Cu > Ni > Pb > Ba > Fe > Cd > Cr > Al > Zn > As > Ti > Mn > Mg. The highest BCF for the concentration of metals in parasites and muscle were Cu and Ni with values of 98.2 and 80.3, respectively, while in the liver the BCF value of Cd was 89.6 (Table 4).

Table 2
Differences between element concentrations in *Acestrorhynchus lacustris* and *Contracaecum* sp. larvae, obtained by ANOVA test with $p \le 0.01$.

Element	*Contracaecum* sp. ↔ liver	*Contracaecum* sp. ↔ muscle	Liver ↔ muscle
Mg	L	M	n.s.
Al	C	C	n.s.
Ti	C	C	n.s.
Cr	C	C	M
Mn	L	C	L
Fe	L	C	L
Ni	C	C	L
Cu	C	C	n.s.
Zn	C	C	L
As	C	C	n.s.
Cd	C	C	M
Ba	C	C	n.s.
Pb	C	C	n.s.

M: muscle; L: liver; C: *Contracaecum* sp.
n.s.: not significant difference by ANOVA.

4. Discussion

In this study, we evaluated the accumulation potential of metals in nematode larvae. Twelve of the thirteen elements analyzed were detected in higher concentrations in *Contracaecum* sp. larvae than in host tissues. We observed that the elements As, Cd, and Pb, known for their high toxicity (Merian et al., 2004), mostly accumulated in the parasites. However, the elements considered to be essential, such as Cu and Zn, were also present in higher concentrations in parasites, except for Mg. These results are similar to those obtained by Nachev et al. (2013) who compared the metal accumulation capacity between *Pomphorhynchus laevis* (Müller, 1776) (Acanthocephala) and larvae *Eustrongylides* sp. (Nematoda) parasitizing *Barbus barbus* (Linnaeus, 1758) in a river in Bulgaria. They also observed that the nematodes showed a higher accumulation capacity for essential elements (Cu, Fe, and Zn).

Several studies analyzing the accumulation capacity in acanthocephalan fish parasites have been conducted in recent decades (Brázová et al., 2012; Brázová et al., 2015; Nachev et al., 2010; Schludermann et al., 2003; Sures and Siddall, 1999; Sures and Siddall, 2003; Sures et al., 1999b; Sures et al., 2005; Thielen et al., 2004). These studies confirmed the role of this phylum as a powerful bioindicator and biomonitor of water pollution. In contrast, there have been fewer studies of nematode species than of acanthocephalans, and the results obtained are often inconclusive and indicate that the parasites have a low accumulation capacity. Otachi et al. (2014), who evaluated the accumulation capacity of *Contracaecum multipapillatum* (Drasche, 1882) compared to the muscle and liver of *Oreochromis leucostictus* (Trewavas, 1933) in a lake in Kenya, found that the concentration of essential and non-essential elements were higher in the host tissues, indicating that this parasite is therefore not an efficient accumulation bioindicator, as it showed a low metal accumulation capacity. Compared to the results of our study, it is clear that a vast range of variables influences the accumulation capacity of metals by the parasites as well as by their hosts.

Numerous variables can influence the accumulation capacity of metals by nematodes compared to other parasites. These variables include parasite development stage, location on host, feeding and excretion mechanisms, and even competition in the host-parasite relationship or between parasites of the same infra-community (Nachev et al., 2013; Otachi et al., 2014; Sures et al., 1999a; Sures, 2003). The high concentration of metals in some parasites, particularly heavy metals, can be attributed to the probable ability to produce certain organometallic compounds containing metals in their structures (Khaleghzadeh-Ahangar et al., 2011).

Most *Contracaecum* sp. larvae analyzed in the present study were collected from the intestine and body cavity, which are sites that typically exhibit the highest metal absorption rates by parasites (Nachev and Sures, 2015; Sures et al., 1999a). This may explain the higher concentrations of these elements in the parasites than in host tissues. Once ingested by fish, the nematode is free or encapsulated in the wall of the stomach or intestine and feeds on the blood and host fluids and can later migrate to other organs, the body cavity, or skeletal muscle (Bird and Bird, 1991; Eiras, 1994; Moravec, 1998).

The larval stage that may have also influenced the high accumulation capacity, as in studies where species of adult nematodes were investigated, the results generally showed that the parasite was not a bioaccumulator (Baruš et al., 2001). Pascual and Abollo (2003) compared the accumulation potential in *Anisakis simplex* adults and larvae (Rudolphi, 1809) in different hosts and in marine environments and observed higher accumulation rates for Cd, Cu, Pb, and Zn in the larvae than in adults. Here, we focused on the third larval stage, which accumulated large amounts of elements, particularly those that are highly

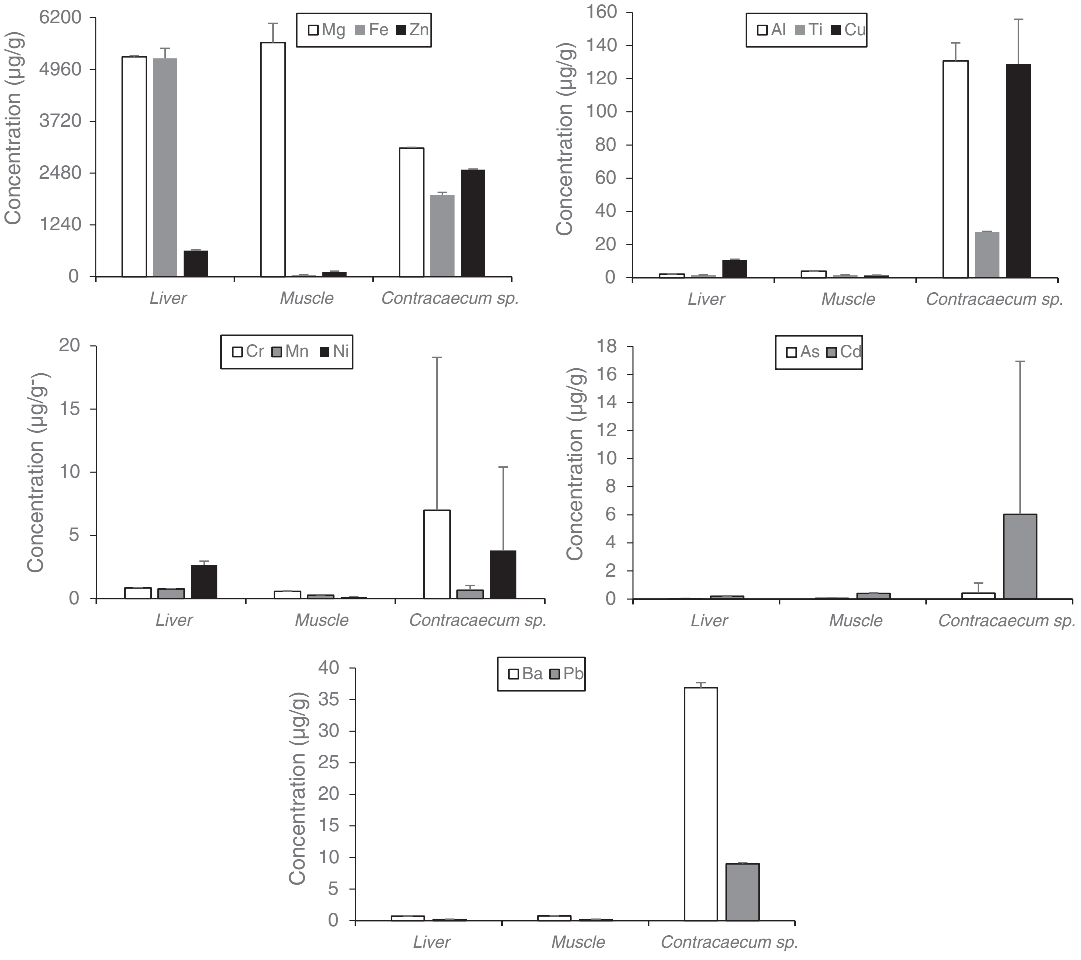

Fig. 1. Average concentration and standard deviation of elements in the organs of *Acestrorhynchus lacustris* and *Contracaecum* sp. larvae.

toxic. This demonstrates that metal uptake begins during an early developmental stage of the parasite because the larvae cuticle is less complex than the adult nematode cuticle (Bird and Bird, 1991), and the larvae are capable of adsorbing metals through the body surface (Nachev et al., 2013), which may result in longer exposure to metals and therefore their accumulation.

5. Conclusion

Our results suggest that *Contracaecum* sp. larvae can be used as bioindicators of metal accumulation because even at an early developmental stage, these organisms show good accumulation ability for the elements considered essential as well as those considered to be toxic (particularly As, Cd, and Pb). Thus, the larvae of *Contracaecum* sp. represent a good tool for monitoring polluted environments.

Table 3
Average element concentrations (µg/g) in *A. lacustris* tissues and *Contracaecum* sp. larvae, obtained by ICP-MS analysis.

Element	Liver	Muscle	*Contracaecum* sp.
Mg	5263.22	5603.18	3077.15
Al	2.14	3.90	130.74
Ti	1.61	1.61	27.54
Cr	0.84	0.56	6.99
Mn	0.76	0.26	0.65
Fe	5226.76	41.99	1951.97
Ni	2.64	0.14	3.81
Cu	10.66	1.34	128.87
Zn	622.66	115.63	2560.70
As	0.03	0.05	0.42
Cd	0.20	0.40	6.03
Ba	0.71	0.75	36.88
Pb	0.17	0.17	8.98

Acknowledgements

The authors would like to thank Ross Martin Thomas for the first editing of the English language and Elsevier's language service for the final revision. To Dr. Beatriz Antoniassi Tavares for help in interpreting

Table 4
Bioconcentration factors $C_{[\mathrm{parasite}]}/C_{[\mathrm{organ}]}$ for *Contracaecum* sp. in the analyzed tissues of *Acestrorhynchus lacustris*.

	$C_{[\mathit{Contracaecum}\ \mathrm{sp.}]}/C_{[\mathrm{organ}]}$ ± SD	
	Liver	Muscle
Mg	0.06 (±0.01)	0.6 (±0.05)
Al	60.9 (±5.4)	33.4 (±2.7)
Ti	17.1 (±5.4)	17.1 (±0.5)
Cr	24.9 (±0.9)	37.7 (±1.4)
Mn	0.9 (±0.03)	2.5 (±0.2)
Fe	0.4 (±0.03)	46.8 (±3.8)
Ni	4.4 (±0.5)	80.3 (±8.3)
Cu	12.1 (±2.6)	98.2 (±27.2)
Zn	4.1 (±0.1)	27.2 (±2.7)
As	45.1 (±15.9)	25.7 (±5.2)
Cd	89.6 (±9.6)	45.7 (±2.9)
Ba	51.8 (±2.0)	49.4 (±1.8)
Pb	56.1 (±13.7)	56.9 (±19.9)

the results related to the heavy metals. We also thank to SISBio for authorizing the collection (40998-2) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2012/23655-0) for financial support.

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