

# *Sclerocollum saudii* Al-Jahdali, 2010 (Acanthocephala: Cavisomidae) as a sentinel for heavy-metal pollution in the Red Sea

## Research Paper

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### Abstract

Currently, fish helminth parasites, especially cestodes and acanthocephalans, are regarded as sentinel organisms to elucidate metal pollution in aquatic ecosystems. Here, 34 specimens of the fish *Siganus rivulatus* were collected in the Red Sea, from a seriously polluted, small lagoon named Sharm-Elmaya Bay, at Sharm El-Sheikh, South Sinai, Egypt; 22 (64.7%) were infected by *Sclerocollum saudii* (Acanthocephala: Cavisomidae). Thus, 22 natural infrapopulations (26–245 individuals) of this parasite were collected from infected fish. Samples of water and sediments from the bay, samples of muscle, intestine and liver from each fish, and samples from the parasite were taken for analysis of heavy metals (cadmium (Cd) and lead (Pb)). Both Cd and Pb concentrations in sediments were higher than those in water. The concentration of these metals were significantly higher in tissues (intestine, liver and muscle) of non-infected fish than those in infected fish, with Pb concentrations consistently higher than those of Cd, and both were drastically decreased in the order: liver > intestine > muscle. Metal concentrations in this acanthocephalan were much higher than those in its fish host. There were strong negative relationships between metal concentrations in tissues (intestine, liver and muscle) of infected fish and infrapopulation size, and between metal concentrations in the acanthocephalan and its infrapopulation size. These relationships strongly suggest competition for these metals between the fish host and its acanthocephalan parasite, and intraspecific competition among acanthocephalan individuals for available metals in the fish intestine. Bioconcentration factors were relatively high, since the mean Cd concentration in *S. saudii* was 239, 68 and 329 times higher than those in intestine, liver and muscle tissues, respectively, of its fish host. Also, mean Pb concentration was 55, 13 and 289 times higher than those in these tissues, respectively. The host–parasite system described here seems to be promising for biomonitoring of metal pollution in the Red Sea.

### Introduction

Environmental parasitology is a recent discipline dealing with the interactions between parasites and pollutants in the environment (Goater *et al.*, 2013; Sures *et al.*, 2017). In this discipline, the use of endohelminth parasites of fish (digenean trematodes, cestodes, nematodes and acanthocephalans) as sentinel organisms to elucidate metal pollution in aquatic ecosystems has received widespread attention (Sures, 2003; Sures *et al.*, 2017). These helminths have a high capacity to accumulate pollutants, such as toxic metals, which are found in very low concentrations in the environment, and to make them more detectable by analytical techniques (Sures *et al.*, 2017). Acanthocephalans and cestodes exhibit the highest accumulation rates compared to other endohelminth parasites (Sures, 2004; Nachev *et al.*, 2013; Sures *et al.*, 2017). Most studies in this field, i.e. using fish helminth parasites as bioindicators for heavy-metal pollution in aquatic ecosystems, were carried out from 1990 to 2017, and mainly focused on host–parasite systems in freshwater ecosystems, but those in marine ecosystems remain little studied (Mazhar *et al.*, 2014; Nachev & Sures, 2016). Also, such studies in the Red Sea region are very rare and, so far, only two studies are known from this region; Bayoumy *et al.* (2008) and Hassan *et al.* (2016) concluded that five monogenean species and four nematode species, respectively, from Red Sea fishes were useful bioindicators for heavy-metal pollution.

The siganid fish *Siganus rivulatus* in the northern Red Sea is parasitized by two acanthocephalan species, *Sclerocollum rubrimaris* and *S. saudii* (Acanthocephala: Cavisomidae) (see Schmidt & Paperna, 1978; Diamant, 1989; Hassanine & Al-Jahdali, 2007; Al-Jahdali *et al.*, 2015). In the present study, this fish was found to be permanently resident and parasitized with *S. saudii* in a seriously polluted, small lagoon named Sharm-Elmaya Bay, at Sharm El-Sheikh, South Sinai, Egypt, so the authors took the opportunity to determine cadmium (Cd) and lead (Pb) concentrations in this host–parasite system to determine its usefulness as a bioindicator for heavy-metal pollution in the Red Sea. Generally, this study is the first

attempt to determine heavy-metal bioaccumulation using a fish–acanthocephalan system from the Red Sea.

## Materials and methods

### Sampling and sample preparation

During March 2017, a sample of 34 specimens of the fish *S. rivulatus* (Teleostei, Siganidae), of nearly the same size (12–15 cm in fork length), were caught by hand net (by scuba-diving) in the Red Sea, from a small lagoon (c. 400 m in diameter and 3–6 m in depth) known as Sharm-Elmaya Bay (27°51.234'N, 34°17.605'E), at Sharm El-Sheikh, South Sinai, Egypt (fig. 1). Water and sediment samples were also taken from six different sites in this bay, which is seriously polluted due to massive tourism, the fleet of motorized boats that occupy the bay and evacuate their waste directly into its water, the antifouling paints used during boat maintenance, and many other maritime and anthropogenic activities (Egyptian Environmental Affairs Agency (EEAA), 2003).

From each fish, samples of dorsal muscle, middle intestine and liver were taken, and kept frozen at  $-30^{\circ}\text{C}$  until further processing for metal analysis. The infrapopulation (all individual worms) of *S. saudii* (Acanthocephala: Cavisomidae) found in the intestine of each infected fish was carefully teased out and counted; ten worms were taken randomly from each infrapopulation as a representative sample, thoroughly homogenized into a composite and kept frozen at  $-30^{\circ}\text{C}$  until further processing for metal analysis. To minimize sample contamination, all the basic precautions (such as using sterilized stainless-steel dissection

instruments, clean plastic vials with lids for preservation of tissue samples and sterile vessels for tissue digestion) were taken during the collection and treatment of samples.

### Metal analysis

Water samples were filtered through a  $0.4\text{-}\mu\text{m}$  membrane filter and acidified with suprapure nitric acid ( $\text{HNO}_3$ ) to pH less than 2, then analysed directly for the heavy metals (Cd and Pb) in an inductively coupled plasma mass spectrometer (ICP-MS; ELAN6100, Perkin Elmer, Concord, Ontario, Canada). Standards and blanks were treated similarly. Metal concentrations in water samples are expressed as  $\mu\text{g l}^{-1}$ .

Sediment samples were analysed according to the method of Oregioni & Aston (1984). In this method, samples were dried in an oven at  $110^{\circ}\text{C}$  for 6 h, and then ground in an agate mortar. One gram of homogenized sample, sieved through a  $0.75\text{-mm}$  sieve, was digested by a mixture of concentrated acids (nitric/perchloric/hydrofluoric acids ( $\text{HNO}_3/\text{HClO}_4/\text{HF}$ ) = 3/2/1). The residue was finally dissolved in 3% hydrochloric acid (HCl; v/v) and its volume made up to 50 ml in a volumetric flask, and then analysed for the heavy metals in the aforementioned instrument. Blank digestions were treated in the same way. Metal concentrations in sediments are expressed as  $\text{mg kg}^{-1}$  dry weight.

Fish and parasite tissue samples were analysed according to the methods of Zimmermann *et al.* (2001) and Nachev (2010). After thawing, 300 mg (wet weight) of the homogenized fish tissues, or 150 mg of parasites, was placed into a 150-ml perfluoralkoxy vessel. A mixture of 2 ml  $\text{HNO}_3$  (65%, suprapure) and 2.5 ml

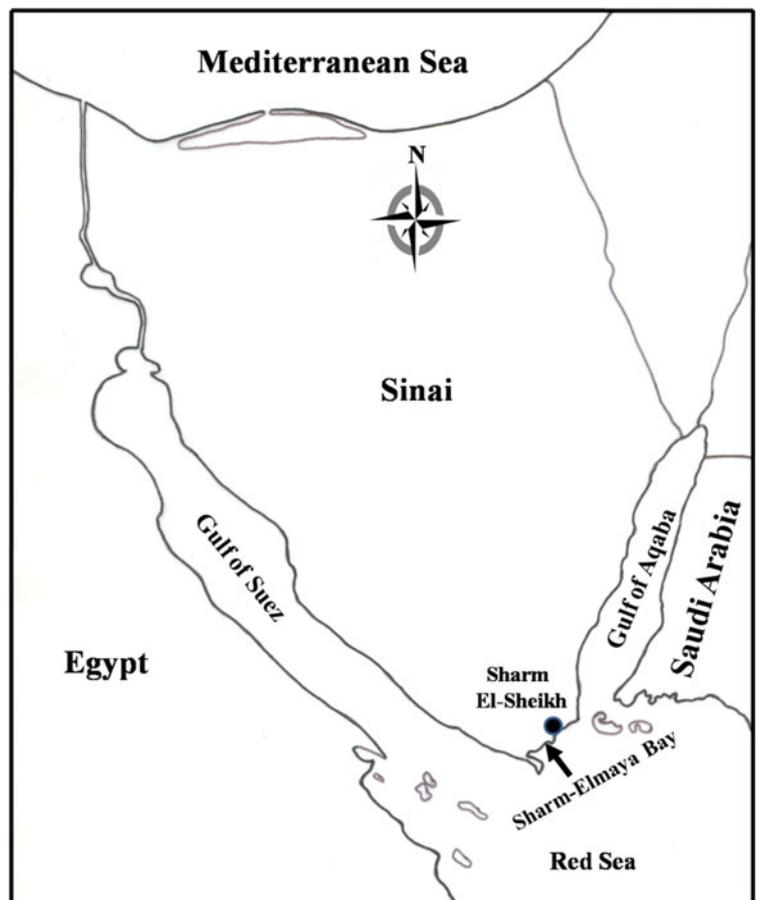


Fig. 1. Map of the Sinai Peninsula, Egypt, showing the location of Sharm-Elmaya Bay.

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%, suprapure) was added and the vessel was heated for 90 min at about 170°C in a microwave digestion system. After digestion, the resulting clear sample solution was diluted to 5 ml with deionized water in a volumetric glass flask, and then analysed for the heavy metals in the aforementioned instrument. Standards and blanks were treated similarly. Metal concentrations in tissues are expressed as mg kg<sup>-1</sup> wet weight.

The quality of analytical procedures was tested using three standard reference materials: (1) CRM-NIST 1640-Trace Elements in Natural Water, National Institute of Standards and Technology, USA; (2) HISS-1-Marine Sediments, National Research Council, Canada; and (3) Dogfish muscle-DORM2, National Research Council, Canada). Analytical blanks were prepared to determine the detection limits.

### Data analysis

Linear regression analyses were used to determine possible relationships between metal concentrations in the different organs of each fish and its acanthocephalan parasite. The statistical package SPSS software (version 19.0 for Windows; SPSS Inc., Chicago, Illinois, USA) was used for data analyses. The bioconcentration factor (BCF) or the ratio of metal concentration in the parasite and the host tissue ( $C_{[parasite]}/C_{[host\ tissue]}$ ) was calculated according to Sures *et al.* (1999).

### Results

Of the 34 *S. rivulatus* examined, 12 (35.3%) were free from any intestinal helminth parasites, while the other 22 (64.7%) were slightly or heavily parasitized by *S. saudii* Al-Jahdali, 2010 (Acanthocephala: Cavisomidae); no other helminth parasites were found in the intestine of these hosts. Accordingly, 22 *S. saudii* infrapopulations, ranging from 26 to 245 individuals, were collected from the infected fish, with a mean intensity of 112.3 (SD: ± 66.0) worms/host. Because fish individuals were nearly equal in size (12–15 cm in fork length), no significant relationship was found between fish size and size of *S. saudii* infrapopulation ( $R^2 = 0.0283$ , slope = 7.236,  $P > 0.713$ ).

The concentrations of Cd and Pb recovered from standard reference materials, accuracy and detection limits of each element are given in table 1.

### Metal (Cd and Pb) concentrations in the bay water and sediments

In all sampling sites, both Cd and Pb concentrations in the sediments were significantly higher than those in the water (table 2), and concentrations of Pb were consistently higher than those of Cd.

### Metal (Cd and Pb) concentrations in tissues of non-infected and infected fish

The Cd and Pb concentrations in the intestine, liver and muscle of 12 non-infected fish are recorded in table 3. In all these tissues, concentrations of Pb were significantly higher than those of Cd, and both decreased in the order: liver > intestine > muscle. There were strong positive relationships between Cd concentrations in fish intestines and its concentrations in both liver and muscle ( $R^2 = 0.7803$ , slope = 2.771,  $P < 0.0001$ ;  $R^2 = 0.9042$ ,

**Table 1.** The concentrations of Cd and Pb in certified reference materials, accuracy and detection limits determined by ICP-MS analyses.

Element	Standard reference material				Accuracy (%)	Detection limit
	CRM-NIST 1640-Trace Elements in Natural Water	HISS-1-Marine Sediments	Dogfish muscle-DORM2			
Cd	Certified value (mg l <sup>-1</sup> )	0.024 ± 0.009	0.043 ± 0.008	0.041 ± 0.001	93.75	0.003
	Recovered value (mg l <sup>-1</sup> )	3.840 ± 0.094	0.023 ± 0.001	0.062 ± 0.002		
Pb	Certified value (mg l <sup>-1</sup> )	3.140 ± 0.04	0.065 ± 0.007	0.065 ± 0.007	94.01	0.006
	Recovered value (mg l <sup>-1</sup> )	11.767 ± 0.105	2.951 ± 0.057	0.062 ± 0.002		
		96.94	93.75	95.34		
		98.01	94.01	95.38		

**Table 2.** Mean concentrations of Cd and Pb in the water and sediments of Sharm-Elmaya Bay (Red Sea), Sharm El-Sheikh, South Sinai, Egypt.

Site	Water ( $\mu\text{g l}^{-1}$ )		Sediment ( $\text{mg kg}^{-1}$ dry wt)	
	Cd	Pb	Cd	Pb
1	0.189 ± 0.015	5.782 ± 0.302	0.492 ± 0.022	16.675 ± 0.735
2	0.167 ± 0.008	6.071 ± 0.981	0.692 ± 0.012	14.833 ± 0.572
3	0.172 ± 0.016	6.563 ± 0.381	0.423 ± 0.013	17.783 ± 0.731
4	0.154 ± 0.012	4.491 ± 0.382	0.605 ± 0.015	19.346 ± 0.362
5	0.142 ± 0.013	5.361 ± 0.241	0.598 ± 0.028	18.994 ± 0.999
6	0.180 ± 0.010	6.409 ± 0.199	0.564 ± 0.014	14.251 ± 0.361
Range	0.142–0.189	4.491–6.563	0.423–0.692	14.251–19.346
Mean ± SD	0.167 ± 0.017	5.779 ± 0.765	0.562 ± 0.094	16.980 ± 2.118

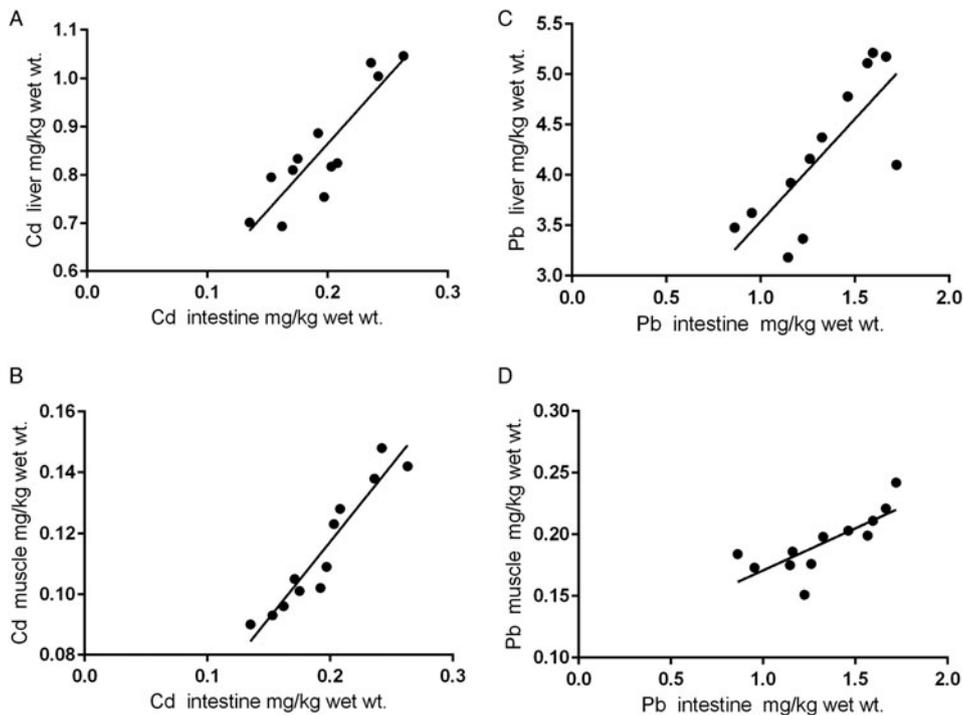
slope = 0.5041,  $P < 0.0001$ , respectively) (fig. 2A, B). Similarly, there were significant positive relationships between Pb concentrations in fish intestines and its concentrations in both liver and muscle ( $R^2 = 0.6024$ , slope = 2.037,  $P = 0.003$ ;  $R^2 = 0.6010$ , slope = 0.0682,  $P = 0.003$ , respectively) (fig. 2C, D). That is, as Cd and Pb concentrations in the intestines of non-infected fish increased, their concentrations in the liver and muscle increased.

Cadmium and Pb concentrations in the tissues of infected fish (table 4) were significantly lower than those in non-infected fish, but the pattern of their accumulation was the same, where Pb concentrations in all tissues were significantly higher than those of Cd, and both were significantly decreased in the order: liver > intestine > muscle. The ranges of Cd concentrations in intestine, liver and muscle of non-infected fish were 0.135–0.263, 0.693–1.046 and 0.090–0.148  $\text{mg kg}^{-1}$  wet wt, respectively, but in infected fish these values were drastically reduced to 0.060–0.142, 0.179–0.460 and 0.044–0.101  $\text{mg kg}^{-1}$  wet wt, respectively.

Similarly, the ranges of Pb concentrations were 0.862–1.721, 3.182–5.214 and 0.150–0.240  $\text{mg kg}^{-1}$  wet wt in the tissues of non-infected fish and were reduced to 0.369–0.801, 1.477–3.277 and 0.063–0.168  $\text{mg kg}^{-1}$  wet wt in the tissues of infected fish. There were strong negative relationships between Cd concentrations in fish intestine, liver and muscle and *S. saudii* infrapopulation size ( $R^2 = 0.7067$ , slope =  $-0.00034$ ,  $P < 0.0001$ ;  $R^2 = 0.7996$ , slope =  $-0.0013$ ,  $P < 0.0001$ ;  $R^2 = 0.8317$ , slope =  $-0.0003$ ,  $P < 0.0001$ , respectively) (fig. 3A–C). Similarly, there were strong negative relationships between Pb concentrations in these tissues and infrapopulation size ( $R^2 = 0.9098$ , slope =  $-0.0017$ ,  $P < 0.0001$ ;  $R^2 = 0.8237$ , slope =  $-0.0074$ ,  $P < 0.0001$ ;  $R^2 = 0.8565$ , slope =  $-0.00044$ ,  $P < 0.0001$ , respectively) (fig. 3D–F). That is, as the infrapopulation size increases, the concentrations of both Cd and Pb in these tissues significantly decrease. Combination of these results strongly suggests competition between the fish host and its acanthocephalan parasites for absorption of these metals.

**Table 3.** Concentrations of Cd and Pb in the selected tissues of 12 non-infected individuals of *Siganus rivulatus*.

Fish no.	Cd ( $\text{mg kg}^{-1}$ wet wt)			Pb ( $\text{mg kg}^{-1}$ wet wt)		
	Intestine	Liver	Muscle	Intestine	Liver	Muscle
1	0.162	0.693	0.096	1.665	5.176	0.221
2	0.197	0.754	0.109	1.261	4.162	0.176
3	0.175	0.833	0.101	1.159	3.921	0.186
4	0.135	0.701	0.090	0.953	3.624	0.173
5	0.208	0.824	0.128	1.325	4.373	0.198
6	0.263	1.046	0.142	1.566	5.111	0.199
7	0.203	0.817	0.123	1.721	4.101	0.242
8	0.242	1.004	0.148	1.462	4.781	0.203
9	0.236	1.032	0.138	0.862	3.479	0.184
10	0.171	0.810	0.105	1.595	5.214	0.211
11	0.153	0.795	0.093	1.224	3.367	0.151
12	0.192	0.886	0.102	1.145	3.182	0.175
Range	0.135–0.263	0.693–1.046	0.090–0.148	0.862–1.721	3.182–5.214	0.150–0.240
Mean ± SD	0.194 ± 0.038	0.849 ± 0.120	0.114 ± 0.020	1.328 ± 0.277	4.207 ± 0.729	0.193 ± 0.024



**Fig. 2.** The relationships between metal concentrations in the intestine and their concentrations in the liver and muscle of non-infected fish. (A) Cd concentrations in intestine vs. its concentrations in liver; (B) Cd concentrations in intestine vs. its concentrations in muscle; (C) Pb concentrations in intestine vs. its concentrations in liver; and (D) Pb concentrations in intestine vs. its concentrations in muscle.

### Metal (Cd and Pb) concentrations in the acanthocephalan parasite

Mean Cd and Pb concentrations were calculated for 22 infrapopulations of *S. saudii* (table 4). These concentrations were much higher than those in the tissues of the fish host, since Cd and Pb concentrations in this acanthocephalan ranged from 14.384 to 32.008 and from 22.36 to 41.450 mg kg<sup>-1</sup> wet wt, respectively. The relationships between Cd or Pb concentrations in the body of *S. saudii* and their concentrations in the fish intestine were clearly positive ( $R^2 = 0.6418$ , slope =  $-0.00235$ ,  $P < 0.0001$ ;  $R^2 = 0.8402$ , slope =  $-0.01964$ ,  $P < 0.0001$ , respectively) (fig. 4A, B), i.e. as the Cd and Pb concentrations in the fish intestine increased, so their concentrations in parasite bodies increased. Controversially, the relationships between Cd or Pb concentrations in *S. saudii* and its infrapopulation size were clearly negative ( $R^2 = 0.8508$ , slope =  $-0.0777$ ,  $P < 0.0001$ ;  $R^2 = 0.6625$ , slope =  $-0.0604$ ,  $P < 0.0001$ , respectively) (fig. 5A, B), i.e. as the infrapopulation size increased the concentration of both Cd and Pb in its individuals significantly decreased. Thus, metal concentrations in this parasite seem largely dependent on those in the host intestine and on its infrapopulation size. Combination of these results strongly suggests intraspecific competition among parasite individuals for absorption of these metals.

Concentrations of Cd and Pb in *S. saudii* were significantly higher than those in the tissues of its fish host (table 4). Thus, bioconcentration factors were relatively high (table 5) and seemed to be highly significant, since the Cd concentration in *S. saudii* was at least about 187-, 56- and 261-fold higher than in fish intestine, liver and muscle, respectively, while the Pb concentration in *S. saudii* was at least about 46-, 11- and 231-fold higher than in these tissues, respectively.

### Discussion

As expected, high concentrations of Cd and Pb were recorded in the acanthocephalan *S. saudii* compared to the tissues of its fish

host. However, tissues of fish infected with this parasite contained significantly lower concentrations of these metals than those of non-infected ones (see below).

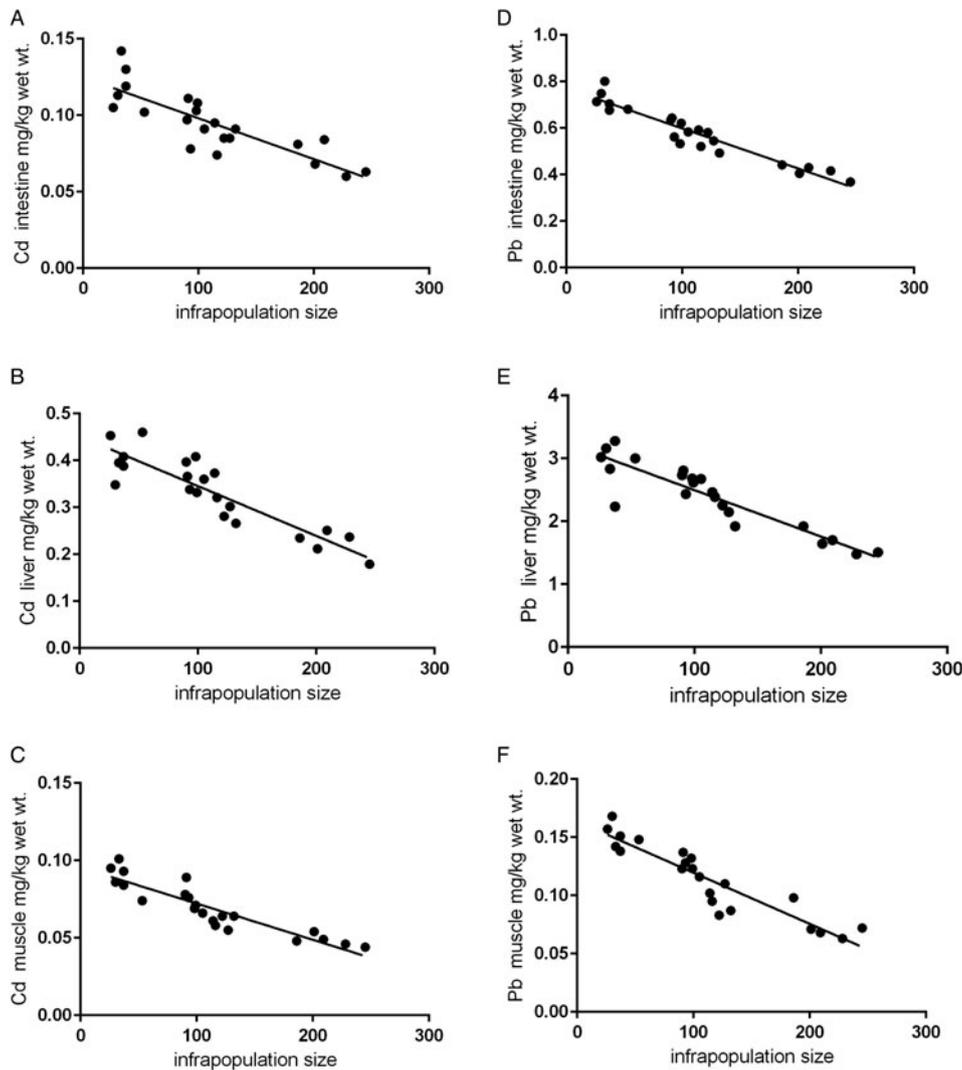
In the present study, the accuracy of analytical techniques ranged from 93 to 98%, which can be considered a reliable analysis.

Various anthropogenic sources (see Egyptian Environmental Affairs Agency (EEAA), 2003) contribute to the serious pollution of Sharm-Elmaya Bay, where Cd and Pb concentrations in the sediments were significantly higher than those in water. High concentrations in sediments may be due to the strong affinity of these metals for particles of bottom sediment or particles of suspended matter that settle on the bottom and build up the bottom sediments (Luorna, 1990; Dauvalter, 1998; Tekin-Ozan & Kir, 2008). Compared to those of the sediment, low metal concentrations in water may be due to the sediment particles and aquatic organisms that accumulate heavy metals from the water column (Karadede & Ünlü, 2000; Al-Saadi *et al.*, 2002; Tekin-Ozan & Kir, 2008).

Our results revealed that Pb concentrations in tissues of non-infected and infected fish were significantly higher than those of Cd, and both metal concentrations decreased in the order: liver > intestine > muscle. Thus, hepatic tissue tends to accumulate higher levels of Cd and Pb than intestinal tissue, while muscle tissue tends to accumulate relatively low metal levels. High concentrations of metals in the liver may be due to their essential role in the synthesis of metallothioneins (metal-binding proteins) that have strong affinities for heavy metals, and concentrate and regulate them in the liver (Buckley *et al.*, 1982; Carpenne & Vašák, 1989; Al-Yousuf *et al.*, 2000; Yousafzai *et al.*, 2009), detoxifying the metal ions (Kojima & Kagi, 1978). Similarly, intestinal tissue is metabolically active and can accumulate heavy metals in high concentrations, as recorded in many fish species (Marzouk, 1994; Deb & Fukushima, 1999; Khalil & Faragallah, 2008; Eneji *et al.*, 2011). Controversially, muscular tissue is less active for heavy metal accumulation (Carpenne & Vašák, 1989; Kargin & Erdem, 1991; Karadede & Ünlü, 1998, 2000; Karadede *et al.*, 2004; Eneji *et al.*, 2011).

**Table 4.** Concentrations of Cd and Pb in 22 infrapopulations of *Sclerocollum saudii* and in the selected tissues of their fish hosts (*Siganus rivulatus*).

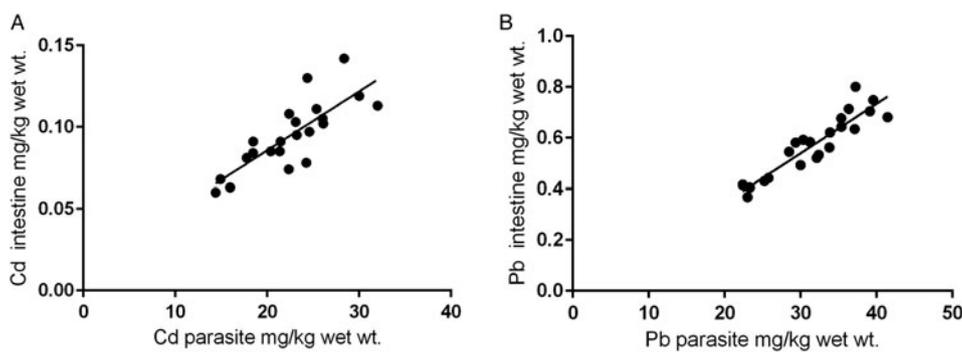
Fish no.	<i>S. saudii</i> infrapopulation size	Cd (mg kg <sup>-1</sup> wet wt)				Pb (mg kg <sup>-1</sup> wet wt)			
		<i>S. saudii</i>	Fish ( <i>S. rivulatus</i> ) tissue			<i>S. saudii</i>	Fish ( <i>S. rivulatus</i> ) tissue		
			Intestine	Liver	Muscle		Intestine	Liver	Muscle
1	26	26.043	0.105	0.453	0.095	36.354	0.714	3.021	0.157
2	30	32.008	0.113	0.348	0.086	39.576	0.749	3.162	0.168
3	33	28.376	0.142	0.395	0.101	37.239	0.801	2.834	0.142
4	37	30.009	0.119	0.388	0.084	39.123	0.705	3.277	0.151
5	37	24.364	0.130	0.408	0.093	35.341	0.677	2.234	0.138
6	53	26.097	0.102	0.460	0.074	41.456	0.681	3.001	0.148
7	90	24.593	0.097	0.397	0.078	37.136	0.635	2.736	0.123
8	91	25.362	0.111	0.366	0.089	35.365	0.644	2.810	0.137
9	93	24.261	0.078	0.338	0.076	33.785	0.562	2.434	0.128
10	98	23.110	0.103	0.408	0.069	32.375	0.533	2.682	0.132
11	99	22.385	0.108	0.332	0.071	33.873	0.621	2.620	0.123
12	105	21.462	0.091	0.360	0.066	31.231	0.584	2.674	0.116
13	114	23.221	0.095	0.373	0.061	30.341	0.592	2.465	0.102
14	116	22.354	0.074	0.321	0.058	32.112	0.521	2.387	0.095
15	122	20.391	0.085	0.281	0.064	29.349	0.581	2.256	0.083
16	127	21.365	0.085	0.302	0.055	28.486	0.545	2.147	0.110
17	132	18.476	0.091	0.266	0.064	29.983	0.493	1.923	0.087
18	186	17.764	0.081	0.235	0.048	25.762	0.442	1.922	0.098
19	201	14.921	0.068	0.212	0.054	23.282	0.405	1.644	0.071
20	209	18.465	0.084	0.251	0.049	25.248	0.430	1.702	0.068
21	228	14.384	0.060	0.237	0.046	22.366	0.416	1.477	0.063
22	245	15.956	0.063	0.179	0.044	23.003	0.369	1.508	0.072
	Range	14.384–32.008	0.060–0.142	0.179–0.460	0.044–0.101	22.36–41.45	0.369–0.801	1.477–3.277	0.063–0.168
	Mean ± SD	22.516 ± 4.653	0.095 ± 0.021	0.332 ± 0.078	0.069 ± 0.017	31.944 ± 5.608	0.577 ± 0.119	2.405 ± 0.536	0.114 ± 0.031



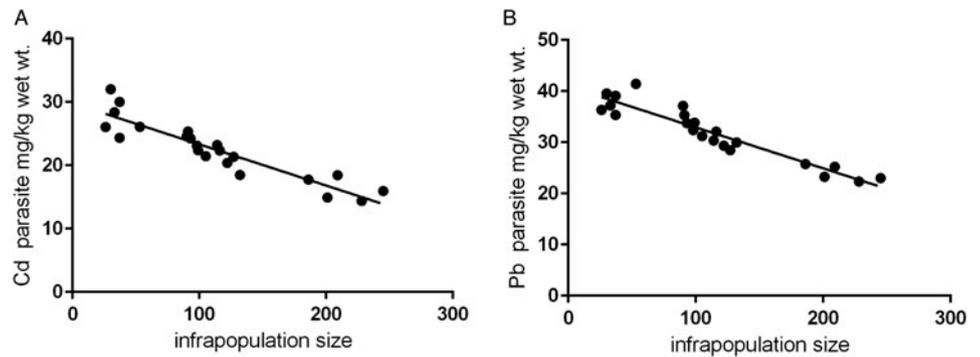
**Fig. 3.** The relationships between metal concentrations in tissues of infected fish and *S. saudii* infrapopulation size in the fish intestine. (A) Cd concentrations in fish intestine vs. infrapopulation size; (B) Cd concentrations in fish liver vs. infrapopulation size; (C) Cd concentrations in fish muscle vs. infrapopulation size; (D) Pb concentrations in fish intestine vs. infrapopulation size; (E) Pb concentrations in fish liver vs. infrapopulation size; and (F) Pb concentrations in fish muscle vs. infrapopulation size.

In the present study, metal concentrations in the tissues of fish infected with the acanthocephalan *S. saudii* were significantly lower than those in non-infected conspecifics. Such a decrease in metal concentration in the tissues of acanthocephalan-infected fish, due to metal uptake by acanthocephalans, has been reported in several studies (e.g. Sures & Siddall, 1999; Sures *et al.*, 1999, 2003; Eira *et al.*, 2009; Brázová *et al.*, 2015; Torres *et al.*, 2015; Paller *et al.*, 2016). However, our study revealed strong negative relationships between metal (Cd or Pb) concentration in tissues of infected fish (intestine, liver, muscle) and *S. saudii*

infrapopulation size, i.e. as the infrapopulation size increases, the concentrations of both Cd and Pb in these tissues significantly decrease. Heavy metals in the aquatic environment are mostly bound to suspended or sediment particles, and only tiny proportions of them are found as free (hydrated) ions and biologically available, i.e. able to be absorbed directly from water by organisms. Biological availability of metals is greatly affected by some environmental factors, such as temperature, salinity, water pH and water hardness (Merian, 2004). Heavy metals can be taken up into fish either through the direct absorption from water by



**Fig. 4.** The relationships between metal concentrations in the fish intestine and their concentrations in the parasite (*S. saudii*). (A) Cd concentrations in the fish intestine vs. its concentrations in the parasite; and (B) Pb concentrations in the fish intestine vs. its concentrations in the parasite.



**Fig. 5.** The relationships between metal concentrations in the parasite (*S. saudii*) and its infrapopulation size in fish intestines. (A) Cd concentrations in parasite vs. its infrapopulation size; and (B) Pb concentrations in parasite vs. its infrapopulation size.

gills or through the ingestion of contaminated food and water, and metal-laden particles, via the alimentary tract, where metal-laden particles are likely to release formerly bound metals due to the lower pH in the intestine (Nachev & Sures, 2016). According to several authors (Grahl, 1990; Hofer & Lackner, 1995; Sures & Siddall, 1999), metals absorbed through the gills or intestinal wall of the fish are carried by blood to different

organs in the body. In the liver, most metals are removed from the blood to form organometallic complexes that then flow through the bile duct into the small intestine, where they can be re-absorbed by the intestinal wall to enter the hepatic-intestinal cycle or excreted with the fish faeces. In acanthocephalan-infected fish, the parasites interrupt the hepatic-intestinal cycle of metals by absorbing organometallic or bile complexes via their tegument

**Table 5.** Bioconcentration factors – BCF ( $= C_{[parasite]}/C_{[host\ tissue]}$ ) – for Cd and Pb in 22 infrapopulations of *Sclerocollum saudii*, calculated with respect to the selected host tissues.

Fish no.	<i>S. saudii</i> infrapopulation size	BCF ( $C_{[parasite]}/C_{[host\ tissue]}$ ) Cd			BCF ( $C_{[parasite]}/C_{[host\ tissue]}$ ) Pb		
		$C_{parasite}/C_{fish\ intestine}$	$C_{parasite}/C_{fish\ liver}$	$C_{parasite}/C_{fish\ muscle}$	$C_{parasite}/C_{fish\ intestine}$	$C_{parasite}/C_{fish\ liver}$	$C_{parasite}/C_{fish\ muscle}$
1	26	248	57	274	50	12	231
2	30	283	91	372	52	12	235
3	33	199	71	280	46	13	262
4	37	252	77	357	55	11	259
5	37	187	59	261	52	15	256
6	53	255	56	352	60	13	280
7	90	253	61	315	58	13	301
8	91	228	69	284	54	12	258
9	93	311	71	319	60	13	263
10	98	224	56	334	60	12	245
11	99	207	67	315	54	12	275
12	105	235	59	325	53	11	269
13	114	244	62	380	51	12	297
14	116	302	69	385	61	13	338
15	122	239	72	318	50	13	353
16	127	251	70	388	52	13	258
17	132	203	69	288	60	15	344
18	186	219	75	370	58	13	262
19	201	219	70	276	57	14	327
20	209	219	73	376	58	14	371
21	228	239	60	312	53	15	355
22	245	253	89	362	62	15	319
	Mean ± SD	239 ± 31	68 ± 9	329 ± 40	55 ± 4	13 ± 1	289 ± 4

with a higher efficiency than the intestinal wall of the fish host. So, the amount of organometallic complexes that are usually re-absorbed by the intestinal wall is markedly reduced in infected fish compared to non-infected conspecifics. Metal uptake through this route by acanthocephalans, and the ability of these worms to reduce metal concentrations in the intestinal wall of their fish hosts, are clear signs of competition between the fish host and its acanthocephalan parasites (Sures, 2002). Bile may play vital roles in the development of acanthocephalans within the fish intestine; it activates the hatching of cystacanths (the infective stage of Acanthocephala) and enhances the absorption of some metals and fatty acids into the acanthocephalan body from the fish intestine (acanthocephalans cannot synthesize their own fatty acids) (Kennedy *et al.*, 1978; Nickol, 1985; Starling, 1985; Sures, 2003).

Concentrations of Cd and Pb in *S. saudii* were much higher than those in the tissues of its fish host. The relationships between the concentrations of these metals in fish intestines and their concentrations in *S. saudii* were clearly positive, i.e. as Cd and Pb concentrations in fish intestine increased, their concentrations in this acanthocephalan increased. Controversially, the relationships between Cd and Pb concentrations in *S. saudii* and its infrapopulation size in fish intestine were clearly negative, i.e. as the infrapopulation size increased, the concentration of both Cd and Pb in its individuals significantly decreased. Decreasing metal concentrations with increasing infrapopulation size strongly suggests intraspecific competition among parasite individuals for absorption of the available metals in the fish intestine (Sures, 2002). Thus, metal concentrations in *S. saudii* seem to be largely dependent on their concentrations in fish intestines and on the parasite infrapopulation size. Intraspecific competition for host resources is common in acanthocephalan infrapopulations in fish (e.g. Sasal *et al.*, 2000; Kennedy, 2006; Poulin, 2006; Al-Jahdali & Hassanine, 2012).

Currently, different fish helminth parasites (digeneans, cestodes, nematodes and acanthocephalans) are regarded as sentinel organisms for the elucidation of metal pollution in aquatic ecosystems (Sures, 2003; Sures *et al.*, 2017). These helminths have a high ability to accumulate pollutants, such as toxic metals that are found in very low concentrations in the environment, and make them more detectable during analytical analysis (Sures *et al.*, 2017). Acanthocephalans and cestodes exhibit the highest accumulation rates (Sures, 2004; Nachev *et al.*, 2013; Sures *et al.*, 2017). In the most well-known example from a freshwater environment, the mean concentrations of Cd and Pb were 400 and 2700 times higher in the acanthocephalan *Pomphorhynchus laevis* than in the muscle tissue of its fish host (*Leuciscus cephalus*), and 27,000 and 11,000 times higher than in the water (Sures *et al.*, 1994; Sures & Taraschewski, 1995). In the present study, mean Cd concentrations in the acanthocephalan *S. saudii* were 239, 68 and 329 times higher than those in the intestine, liver and muscle tissues, respectively, of its fish host. Also, mean Pb concentrations were 55, 13 and 289 times higher than those in these tissues. Such bioconcentration factors are relatively high and seemed to be highly significant, since in marine ecosystems only tiny proportions of trace metals are found as free (hydrated) ions, and hence these metals are biologically less available for uptake by the intestinal parasites compared with freshwater ecosystems, as the concentration of hydrated ions decreases with increasing salinity (Merian, 2004).

Generally, the present host–parasite system seems promising for biomonitoring of metal pollution in the Red Sea. However, *S. saudii*

markedly reduced the mean concentrations of Cd and Pb in the muscle tissue of its fish host from 0.114 and 0.193 mg kg<sup>-1</sup> wet wt in non-infected fish to 0.069 and 0.114 mg kg<sup>-1</sup> wet wt in infected fish. Such a reduction is certainly of benefit for both fish and humans, since it lowers the toxic effects of these metals in fish muscle and makes muscle less contaminated when eaten by a human, since muscle is the most edible part of the fish.

Some worms of *S. saudii* were dead and seen hanging out of the anus of many fish. According to Al-Jahdali *et al.* (2015), the life span of this acanthocephalan in the intestine of its fish host, *S. rivulatus*, ranges from 42 to 52 days. Thus, during this period, *S. saudii* individuals absorb some heavy metals from the intestine of their fish host, and then die and pass out through the anus. Their subsequent degeneration in water releases the metals, which then re-enter the aquatic environment.

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