Article

Acanthocephalan Worms Mitigate the Harmful Impacts of Heavy Metal Pollution on Their Fish Hosts

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Abstract: Toxic metal pollutants in aquatic environments and infestation with intestinal helminths adversely affect the fish health, as well as fish consumers. Acanthocephalan worms in fish intestine have a high potential to absorb and bioaccumulate different heavy metals, especially toxic ones, from the intestine via their tegument with greater efficiency than the fish intestinal wall. Herein, 47 specimens of the fish *Siganus rivulatus* were trapped in the Red Sea, Egypt, from a chronically polluted bay. All were intoxicated with Cd and Pb; 20 (42.5%) were uninfected with any intestinal worm, but the other 27 (57.5%) were infected only by the intestinal acanthocephalan *Sclerocollum rubrimaris*. The number of individual worms in a fish host (intrapopulation size) ranged from 32 to 236. As a reference group, 22 uncontaminated–uninfected specimens of *S. rivulatus* were trapped from a small unpolluted bay. Our results revealed that infection with acanthocephalans alleviates the harmful effects of toxic metals on their fish hosts by: (1) lowering the elevated concentrations of both Cd and Pb in fish liver; (2) lowering the elevated levels of liver enzymes (ALT, AST, ALP, and GGT), glucose, triglycerides, and urea in fish blood serum; and (3) raising the declined levels of total protein and albumin in fish blood serum. All of these were dependent on *S. rubrimaris* intrapopulation size in fish intestine.

Keywords: *Sclerocollum rubrimaris*; *Siganus rivulatus*; cadmium; lead; bioaccumulation; biochemical parameters

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1. Introduction

Undoubtedly, fish health is adversely affected by pollution and helminth parasites. Contamination of aquatic environments with toxic heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) leads to their accumulation in the different organs of fish, such as gills, intestine, liver, kidneys, and muscle [1,2]. This accumulation can cause severe tissue damage, various biochemical and functional disorders, and serious chronic diseases in these organs [3–6]. Liver is one of the major metabolically active organs in the body and is the main detoxification site, and its tissue tends to accumulate higher levels of heavy metals than any other tissues in the body [7–13]. Acanthocephala is a small phylum of mesoparasitic and gonochoristic worms. The adults are extremely specialized to live in the digestive tract of vertebrates, especially fish, where they use their armed proboscis to attach to the intestinal mucosa or to the intestinal wall. However, these worms have no digestive tract and absorb their nutrients through their tegument [14]. Generally, acanthocephalans adversely affect and damage the nature of intestinal tissue at the attachment sites, causing severe histopathological changes [15–22].

Over the years 1995–2020, a large number of valuable studies have demonstrated that fish helminth parasites, especially those that do not have a digestive tract (i.e., acanthocephalans and cestodes), actively use their tegument to absorb nutrients from the fish's intestine. Thus, these helminths are good sentinels for trace metal pollution in aquatic environments

since they have much higher potential and capacity than their fish hosts to bioaccumulate different metals, especially toxic ones, from the fish's intestine [13,23–29]. This process may affect the physiology of the fish hosts by lowering the amount of metals available in their intestine before absorption through the intestinal wall to other organs. If these metals are toxic, then the parasites have a beneficial impact on their fish hosts, by minimizing the amounts of these metals in their tissues [13,23,30,31]. Confirming this, several studies have shown that metal concentrations in the tissues of acanthocephalan-infected fish were significantly less than those of uninfected conspecifics [13,28,32–35].

In fishes, the levels of blood biochemical parameters are commonly used to assess their health status. For example, the levels of serum liver enzymes (alanine amino transferase ALT, aspartate amino transferase AST, alkaline phosphatase ALP, and gamma-glutamyl transferase GGT) are used to assess the liver functions and hepatocyte integrity [36,37]. The levels of serum glucose and triglycerides are used to assess the processes of energy metabolism [38,39], and the levels of serum total protein STP, albumin, and urea are used to assess the processes of protein metabolism and growth [40,41]. These parameters are sensitive, and their levels significantly altered in fishes living in metal-polluted environments, leading to serious health problems and diseases [42–45]. Additionally, several studies [46–51] have demonstrated that fish infection with helminth parasites significantly increases the levels of blood biochemical parameters.

The siganid fish *Siganus rivulatus* is common and an important fishery target in the Red Sea and is specifically parasitized by the acanthocephalan *Sclerocollum rubrimaris* Schmidt and Paperna, 1978 [28,52–54]. It is economically important, easy to obtain, and the only fish parasitized with acanthocephalans. In the present study, this host–parasite system was found resident in a chronically polluted small bay named “Boat Harbour”, at Sharm El-Sheikh, South Sinai, Egypt, and was used to estimate the acanthocephalans' uptake of toxic metals from the intestine of their fish hosts and to assess for the first time the corresponding impacts of this uptake on some biochemical parameters in these hosts (liver function parameters, energy metabolism parameters, and protein metabolism parameters), and whether these impacts were harmful or beneficial to these hosts.

2. Materials and Methods

2.1. Ethics

This work complied with the current Egyptian regulations of Institutional Animal Care and Use Committee (local IACUC–Cairo University, Cairo, Egypt: approval code, CU/F/8365: 7/1/2020), the Egyptian universities guidelines for the care of experimental animals, and with the Egyptian Environmental Affairs Agency (EEAA).

2.2. Samples Collection and Preparation

During February of 2020, a sample of 47 specimens of the teleost fish *Siganus rivulatus* (Siganidae), approximately with equal sizes (20.87 ± 1.83 cm in total length), were trapped alive by a small conical fishing hand net (during scuba diving) in the Red Sea, from a small bay named “Boat Harbour” ($27^{\circ}51.2' \text{ N}$, $34^{\circ}17' \text{ E}$), at Sharm El-Sheikh, South Sinai, Egypt (Figure 1). This bay is chronically or severely polluted due to the huge discharge of untreated sewage and other wastes from a large number of motorboats directly into this bay. However, and during the same month, 22 specimens of this fish, also with nearly equal sizes (20.14 ± 2.10 cm in total length), were taken alive from a small uninhabited and clear bay near Safaga (133 km south of Sharm El-Sheikh) (Figure 1), far away from anthropogenic pollution sources.

To detect the metal pollution in the two bays, 5 sets of water samples, each composed of 3 replicates (100 mL), were taken from different sites in each bay. Each replicate was filtered and preserved with 2 mL of HNO_3 . Within 40 min after trapping, fishes were anesthetized with benzocaine, and a blood sample of 10 mL was taken aseptically from the caudal vein of each fish via a 10 mL disposable syringe; this step was carried out quickly to reduce fish stress. Then, the blood sample was transferred into a blood collection tube (Becton

Dickinson, Vacutainer Plus Serum Blood Collection Tube, 6 mL), left undisturbed and allowed to clot for 30 min at room temperature (25 °C). Finally, the sample was centrifuged (Eppendorf 5810 R, Hamburg, Germany) at 2000 rpm for 10 min, and the formed serum was immediately transferred into a clean polypropylene tube. The collected blood sera were stored frozen at −25 °C until being processed for various biochemical analyses.

Figure 1. A map showing the location of Sharm El-Sheikh, South Sinai, Egypt, and Safaga (133 km south of Sharm El-Sheikh).

After dissection, a sample was taken from the liver of each fish and kept frozen at −20 °C until being processed for metal analysis. All individuals (intrapopulation) of *Sclerocollum rubrimaris* Schmidt et Paperna, 1978, found in the intestine of each infected fish were carefully teased out and counted; 20 worms were haphazardly taken from each infected fish as a representative sample of this parasite, perfectly homogenized into a composite, and then kept frozen at −20 °C until being processed for metal analysis.

To minimize probability of sample contamination, standard precautions such as using highly sterilized stainless steel dissection instruments, highly sterilized vessels for tissues digestion, high-quality analytical reagents, and clean plastic vials with lids for preservation of tissues and samples were followed during the collection and treatment of samples.

2.3. Samples Analyses

Seawater samples were filtered through a 0.4µm membrane filter and acidified with nitric acid 65% Suprapur® to pH less than 2, then analyzed directly for the heavy metals (Cd and Pb) in Perkin Elmer Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; ELAN6100, Concord, ON, Canada). Metal concentrations in water samples are expressed as µg L^{−1}.

Three replicates from each collected blood serum were used to estimate some liver function parameters (the enzymes alanine amino transferase ALT, aspartate amino transferase AST, alkaline phosphatase ALP, gamma-glutamyl transferase GGT), some energy metabolism parameters (glucose and triglycerides), and some protein metabolism parameters (serum total protein STP, albumin and urea) in all the examined fishes. All these parameters were measured using the AU5800 Automated Clinical Chemistry Analyzer (Beckman Coulter Inc., Brea, CA, USA); ALT, AST, ALP, GGT, glucose, triglycerides, STP, albumin and urea were measured at wavelengths of 340, 340, 450, 440, 360, 680, 700, 600, and 340 nm.

Three replicates from each fish liver sample or from each parasite composite sample were analyzed for heavy metals (Cd and Pb) as recommended by Zimmermann et al. [55] and Nachev [56]. After thawing, 150 mg (wet weight) of homogenized fish liver or 40 mg of the parasite composite was placed in 150 mL perfluoralkoxy (PFA) vessel. For sample digestion, 2 mL of nitric acid 65% Suprapure® and 2.5 mL of hydrogen peroxide 30% Suprapure® were mixed and the mixture was poured into the vessel, which was heated for 90 min. at about 170 °C in a microwave digestion system (CEM GmbH, Kamp-Lintfort, Germany; Model MDS). The obtained solution was diluted to 5 mL Molecular Grade Water™ in a volumetric glass flask, and then analyzed for heavy metals in the above-named instru-

ment. Metal concentrations in the tissue samples are expressed as mg kg^{-1} wet weight. Two standard reference materials were used to test the accuracy of ICP-MS analyses: (1) SRM-NIST 1640-Trace Elements in Natural Water (National Institute of Standards and Technology, Gaithersburg, MD, USA), and (2) Dogfish liver-DOLT-5 (National Research Council, Ottawa, ON, Canada).

2.4. Data Analysis

Parasitological terms used in this study follow those of previous studies [57,58]. Confidence intervals (CI) were used to estimate the certainty of mean intensity of infection and mean abundance of parasite. Duncan's Multiple Range Test [59] was applied to test for differences between levels of liver enzymes, other blood parameters, and Cd and Pb concentrations in the worm and its host. Linear regression analyses were used to determine possible relationships between metal concentrations in both fish liver and worm and worm infrapopulation size in fish intestine or between levels of liver enzymes (ALT, AST, ALP and GGT), energy metabolism parameters (glucose and triglycerides), or protein metabolism parameters (total protein STP, albumin and urea) in fish blood serum and worm infrapopulation size in fish intestine. The Graph Pad PRISM 7.0 statistical package (GraphPad Software, San Diego, CA, USA) was used for data analyses.

3. Results

Cadmium and lead concentrations recovered from the certified reference materials, the accuracy or the quality of the analytical technique, and the detection limits of each metal are shown in Table 1.

Table 1. Cd and Pb concentrations in standard reference materials and accuracy and detection limits determined by ICP-MS analyses.

Metal	Standard Reference Materials							
	SRM-NIST 1640-Trace Elements in Natural Water				Dogfish Liver DOLT-5			
	Certified Value (mg L^{-1})	Recovered Value (mg L^{-1})	Accuracy (%)	Detection Limit (mg L^{-1})	Certified Value (mg/km)	Recovered Value (mg/km)	Accuracy (%)	Detection Limit (mg/km)
Cd	3.961 ± 0.072	3.904 ± 0.016	98.56	0.007	14.5 ± 0.400	14.26 ± 0.019	98.34	0.004
Pb	12.005 ± 0.040	11.550 ± 0.014	96.20	0.008	0.16 ± 0.036	0.154 ± 0.022	96.25	0.006

3.1. Metal Pollution and Intestinal Helminth Parasites of Fishes in the Two Bays

Heavy metals including Cd and Pb were undetectable in all water and fish liver samples taken from the small uninhabited bay at Safaga, which appeared to have clean natural environment. However, no intestinal helminth parasites were observed in the examined fishes taken from this bay; these fishes seemed to be healthy, and were therefore considered as a "reference group", i.e., "uncontaminated-uninfected fishes". In contrast, Cd and Pb were readily detectable in all water and fish liver samples taken from the chronically polluted Boat Harbour, where mean Cd and Pb concentrations in its water were 0.138 ± 0.006 and 1.525 ± 0.005 , respectively (see below). Of the 47 examined fishes from this bay, 20 (42.5%) were uninfected with any intestinal worm, i.e., "contaminated-uninfected fishes", while the other 27 (57.5%) were slightly or intensely infected by an intestinal acanthocephalan worm named *Sclerocollum rubrimaris* Schmidt et Paperna, 1978, i.e., "contaminated-infected fishes"; no other intestinal worms were found in the examined fishes. The infrapopulation size of *S. rubrimaris* ranged from 32 to 236 individuals/fish host, with a mean intensity of 134.29 ± 61.9 worms/ fish host (95% CI = 75.80, 198.77) and mean abundance of 77.14 worm/fish (95% CI = 74.48, 79.79).

3.2. Serum Biochemical Parameters in Uncontaminated-Uninfected Fishes (Reference Group)

In their blood sera, the mean levels of liver enzymes, ALT, AST, ALP, and GGT were 60.65 ± 3.26 , 77.76 ± 4.02 , 43.02 ± 2.88 , and 8.46 ± 1.26 U/L, respectively (Table 2), and mean levels of other serum biochemical parameters, glucose, triglycerides, total protein, albumin and urea were 66.09 ± 2.80 mg/dL, 114.29 ± 3.20 mg/dL, 22.32 ± 2.76 g/L,

10.46 ± 01.76 g/L, and 28.45 ± 1.66 mg/dL, respectively (Table 3). These values were considered herein as the means of baseline levels since they were measured in uncontaminated–uninfected fishes living in a natural, unpolluted environment.

Table 2. Cd and Pb concentrations and levels of serum liver enzymes (ALT, AST, ALP and GGT) in the reference group, and in the contaminated–uninfected and contaminated–infected individuals of the fish *Siganus rivulatus*.

Fish	No. Worms in Fish Intestine (Infrapopulation Size)	Metal in Fish Liver (mg kg ⁻¹ Wet Weight)		Fish Serum Parameter (Enzymes)				
		Cd	Pb	ALT(U/L)	AST(U/L)	ALP(U/L)	GGT (U/L)	
Reference group (n = 22)	-	Range	Not detectable	Not detectable	55.28–65.64	71.25–84.76	38.78–46.83	6.30–10.70
		Mean ± SD	-	-	60.65 ± 3.26	77.76 ± 4.02	43.02 ± 2.88	8.46 ± 1.26
Contaminated–uninfected (n = 20)	-	Range	0.196–0.367	1.588–3.168	81.82–110.08	98.45–126.45	53.24–86.92	10.03–16.86
		Mean ± SD	0.267 ± 0.046	2.278 ± 0.46	96.02 ± 7.47	115.04 ± 7.43	67.54 ± 10.90	13.64 ± 2.17
Contaminated–infected (n = 27)	32–236	Range	0.088–0.197	0.943–1.912	72.01–93.28	81.02–108.16	45.11–71.32	7.15–12.93
		Mean ± SD	0.143 ± 0.02	1.47 ± 0.21	80.35 ± 6.88	91.31 ± 8.46	58.58 ± 7.43	10.31 ± 1.62

Table 3. Cd and Pb concentrations and levels of serum glucose, albumin, total protein STP, and triglycerides in the reference group, in the contaminated–uninfected, and in the contaminated–infected individuals of the fish *Siganus rivulatus*.

Fish	No. Worms in Fish Intestine (Infrapopulation Size)	Metal in Fish Liver (mg kg ⁻¹ Wet Weight)			Fish Serum Parameter				
		Cd	Pb		Glucose (mg/dL)	Triglycerides (mg/dL)	Total Protein (g/L)	Albumin (g/L)	Urea (mg/dL)
Reference group (n = 22)	-	Range	Not detectable	Not detectable	62.72–70.73	109.23–117.83	17.65–26.52	7.97–13.77	26.21–31.25
		Mean ± SD	-	-	66.09 ± 2.80	114.29 ± 3.20	22.32 ± 2.76	10.46 ± 01.76	28.45 ± 1.66
Contaminated–uninfected (n = 20)	-	Range	0.196–0.367	1.588–3.168	102.67–125.48	148.49–172.53	12.15–16.53	4.08–7.20	42.34–55.27
		Mean ± SD	0.267 ± 0.046	2.278 ± 0.46	112.15 ± 6.87	161.66 ± 7.95	14.44 ± 1.28	6.97 ± 0.90	49.31 ± 3.70
Contaminated–infected (n = 27)	32–236	Range	0.088–0.197	0.943–1.912	79.80–100.71	126.35–151.03	16.57–20.53	6.32–10.45	33.06–45.48
		Mean ± SD	0.14 ± 0.02	1.47 ± 0.21	88.82 ± 6.77	136.66 ± 6.97	18.57 ± 1.23	8.56 ± 1.20	38.09 ± 3.71

3.3. Serum Biochemical Parameters in Contaminated-Uninfected Fishes

Mean Cd and Pb concentrations in the livers of these fishes were high (0.267 ± 0.04 and 2.278 ± 0.46 mg kg^{−1} wet weight, respectively) (Table 2). Compared to those of the reference group, mean levels of serum liver enzymes (ALT, AST, ALP, and GGT) were significantly higher ($p < 0.05$) (Table 2). Similarly, mean levels of serum glucose and triglycerides were significantly higher ($p < 0.05$), while mean levels of serum total protein and albumin were significantly lower ($p < 0.05$), but mean level of serum urea was significantly higher ($p < 0.05$) (Table 3). These results point to significant changes or abnormalities in serum biochemical parameters of contaminated–uninfected fishes.

3.4. Serum Biochemical Parameters in Contaminated-Infected Fishes

Mean Cd and Pb concentrations in the livers of these fishes (0.143 ± 0.02 and 1.47 ± 0.21 mg kg^{−1} wet weight, respectively) were lower than those in contaminated–uninfected fishes (Table 2). Contaminated–infected fishes were organized according to the total number of *S. rubrimaris* in each fish intestine (infrapopulation size) (Table 4). Mean concentrations of Cd and Pb in the worm (13.147 ± 2.51 and 20.11 ± 3.70 mg kg^{−1} wet weight, respectively) were significantly much higher than those in the livers of their fish hosts ($p < 0.05$) (Table 4), i.e., the acanthocephalan worm *S. rubrimaris* bioconcentrates Cd and Pb with about 91 and 13-fold higher than in fish liver. Negative relationships were found between Cd or Pb concentrations in fish liver and worm infrapopulation size ($R^2 = 0.8967$, $p < 0.0001$; $R^2 = 0.7695$, $p < 0.0001$, respectively) (Figure 2a,b), i.e., as the worm infrapopulation size in fish intestine increased the concentration of both Cd and Pb in fish liver decreased. Additionally, clear negative relationships were found between Cd or Pb concentrations in the worm and its infrapopulation size ($R^2 = 0.7480$, $p < 0.0001$; $R^2 = 0.7812$, $p < 0.0001$, respectively) (Figure 2c,d), i.e., as the worm infrapopulation size increased, both Cd and Pb concentrations in its individuals significantly decreased. Thus,

metal concentrations in the fish liver and in the worm appear to be primarily dependent on worm infrapopulation size in fish intestine.

Table 4. Cadmium and Pb concentrations in 27 infrapopulations of the acanthocephalan worm *S. rubrimaris* and in the liver of their contaminated fish hosts (*Siganus rivulatus*).

No. Fish	No. Worms in Fish Intestine (Infrapopulation Size)	Cd (mg kg ⁻¹ Wet Weight)		Pb (mg kg ⁻¹ Wet Weight)	
		Fish Liver	Worm	Fish Liver	Worm
1	32	0.189	14.268	1.834	21.453
2	45	0.163	15.762	1.673	24.876
3	56	0.171	18.193	1.776	23.172
4	63	0.197	16.342	1.611	26.382
5	69	0.165	17.872	1.912	25.371
6	75	0.178	16.237	1.732	24.746
7	81	0.164	16.214	1.602	26.136
8	86	0.174	15.438	1.504	25.258
9	91	0.158	14.984	1.588	22.313
10	93	0.161	13.123	1.474	23.459
11	105	0.147	14.872	1.501	18.402
12	113	0.152	12.457	1.421	20.836
13	124	0.142	13.741	1.534	21.238
14	133	0.148	11.763	1.488	19.418
15	139	0.154	10.571	1.507	18.472
16	147	0.138	12.234	1.358	17.943
17	159	0.132	11.873	1.523	16.634
18	164	0.148	11.243	1.440	18.391
19	172	0.124	12.384	1.389	19.146
20	177	0.130	10.236	1.483	17.541
21	182	0.119	11.763	1.301	15.382
22	199	0.112	10.505	1.285	16.637
23	218	0.105	10.652	1.375	15.812
24	216	0.114	9.894	0.943	16.211
25	222	0.088	11.611	1.189	15.533
26	229	0.106	9.921	1.201	16.432
27	236	0.097	10.841	1.126	15.961
Range		0.088–0.197	9.894–18.193	0.943–1.912	15.382–26.382
Mean ± SD		0.143 ± 0.02	13.147 ± 2.51	1.47 ± 0.21	20.12 ± 3.70

Figure 2. Cont.

Figure 2. The relationships between metal concentrations in fish liver or in worm body and worm infrapopulation size in fish intestine: (a) Cd concentrations in fish liver vs. worm infrapopulation size, (b) Pb concentrations in fish liver vs. worm infrapopulation size, (c) Cd concentrations in worm body vs. its infrapopulation size, (d) Pb concentrations in worm body vs. infrapopulation size.

The mean levels of serum liver enzymes, ALT, AST, ALP, and GGT in contaminated-infected fishes were significantly lower than those in contaminated-uninfected fishes ($p < 0.05$) (Table 2), and clear negative relationships were found between levels of these enzymes and worm infrapopulation size ($R^2 = 0.744$, $p < 0.0001$; $R^2 = 0.8309$, $p < 0.0001$; $R^2 = 0.8834$, $p < 0.0001$; $R^2 = 0.8675$, $p < 0.0001$, respectively) (Figure 3a–d). Similarly, mean levels of serum glucose and triglycerides in contaminated-infected fishes (Table 3) were significantly lower than those in contaminated-uninfected fishes ($p < 0.05$), and clear negative relationships were found between these levels and worm infrapopulation size ($R^2 = 0.8618$, $p < 0.0001$; $R^2 = 0.8445$, $p < 0.0001$) (Figure 4a,b). However, mean levels of serum total protein and albumin in contaminated-infected fishes were higher than those in contaminated-uninfected fishes (Table 3), and clear positive relationships were found between these levels and worm infrapopulation size ($R^2 = 0.7609$, $p < 0.0001$; $R^2 = 0.7697$, $p < 0.0001$) (Figure 4c,d). Moreover, mean level of serum urea in contaminated-infected fishes was significantly lower than that in contaminated-uninfected fishes ($p < 0.05$) (Table 3), and a clear negative relationship was found between this level and worm infrapopulation size ($R^2 = 0.8339$, $p < 0.0001$) (Figure 4e).

Figure 3. Cont.

Figure 3. The relationships between levels of serum liver enzymes and worm infestation size in fish intestine: (a) ALT level in fish serum vs. worm infestation size, (b) AST level in fish serum vs. worm infestation size, (c) ALP level in fish serum vs. worm infestation size, (d) GGT level in fish serum vs. worm infestation size.

Figure 4. Cont.

Figure 4. The relationships between levels of selected biochemical parameters in fish blood serum and worm infestation size in fish intestine: (a) glucose level in fish serum vs. worm infestation size, (b) triglycerides level in fish serum vs. worm infestation size, (c) total protein level in fish serum vs. worm infestation size, (d) albumin level in fish serum vs. worm infestation size, (e) urea level in fish serum vs. worm infestation size.

4. Discussion

In the current study, the accuracy of the analytical procedures carried out by the Coupled Plasma-Mass Spectrometer (ICP-MS) was about 96%, which can be considered a reliable analysis.

In aquatic environments contaminated with toxic metals (e.g., Cd, Pb, Hg, As), the accumulation of these metals in the different organs of fish adversely affects its health by causing severe tissue damage, various biochemical and functional disorders, and serious chronic diseases in these organs (3–6). Similarly, acanthocephalan worms, due to their armed proboscises, adversely affect the health of their fish hosts by damaging the nature of intestinal tissue in these hosts at the attachment sites, causing severe histological and histopathological changes [15–22]. In contrast, several studies [13,23,24,31] have pointed to a beneficial impact of acanthocephalans on their fish hosts because these worms have much higher potential and capacity than their fish hosts to bioaccumulate different metals, especially the toxic ones, and thus can minimize the amounts of heavy metals in the tissues of these fish hosts. However, numerous studies [13,26–29,32,34] revealed that metal concentrations in the tissues of acanthocephalan-infected fish were significantly less than those of uninfected conspecifics. In aquatic environments, heavy metals are generally bound to suspended particles or adsorbed on particulate organic matter, and only minute portions of them are present in water as free ions (hydrated) and identified as “biologically available metals”, i.e., can be taken up directly from water by living organisms. These portions are affected by several environmental factors, such as water salinity, temperature, pH, etc. [60]. Fishes absorb heavy metals through their gills or intestinal wall, and in the liver, most metals are removed from blood to incorporate in organometallic complexes carried by bile into the small intestine, where these complexes can be re-absorbed through the intestinal wall into the general circulation or eliminated in fish feces [23,61,62]. Acanthocephalans interfere with this re-absorption and absorb organometallic or bile complexes via their tegument with greater efficiency than the intestinal wall of the fish host [23,63]. Thus, the amounts of bile complexes that are usually re-absorbed by the fish intestinal wall are significantly reduced. Our results showed that metal (Cd or Pb) concentrations in the liver of fish infected with *S. rubrimaris* were distinctly lower than those in uninfected conspecifics. This decline in metal concentrations due to their uptake by acanthocephalans has been recorded in numerous studies [13,23,24,28,32,33,35,64–66]. However, our results revealed that *S. rubrimaris* bioconcentrates Cd and Pb at levels about 91- and 13-fold higher than in fish liver, i.e., this worm has a high ability or capacity to easily absorb and bioaccumulate considerable amounts of these metals from fish intestine. Thus, the present host–parasite

system, *Siganus rivulatus*–*Sclerocollum rubrimaris* seems to be very useful for biomonitoring trace metal pollution in the Red Sea. Additionally, our results revealed clear negative relationships between Cd or Pb concentrations in the liver of infected fish and *S. rubrimaris* infrapopulation size, i.e., as the worm infrapopulation size increases in fish intestine, the concentrations of both Cd and Pb in fish liver significantly decrease. Moreover, there were clear negative relationships between Cd and Pb concentrations in *S. rubrimaris* and its infrapopulation size, i.e., as the worm infrapopulation size in fish intestine increased, the concentration of both Cd and Pb in its individuals significantly decreased. This decrease firmly suggests intraspecific competition among *S. rubrimaris* individuals for absorption the available amounts of these metals from fish intestine. This pattern of competition for host intestinal resources is well known in acanthocephalan infrapopulations [13,28,63,67,68].

In ecotoxicology, serum liver enzymes such as ALT, AST, ALP, and GGT are commonly used as sensitive biomarkers to elucidate the impact of water pollutants on fish health [69–71]. ALT and AST are essential amino acid-metabolizing enzymes in the liver of teleost fishes [36,37,72,73], ALP is contained in a membrane-bound vacuoles in hepatocytes and osteocytes and plays a vital role [36], and GGT is an abundant enzyme in hepatocytes and plays an essential role in xenobiotic detoxification [36]. Serum levels of these enzymes are mostly increased in fishes exposed to heavy metal pollution [45,74–78]. This increase may be due to the toxic impact of these metals on fishes and points to histological damage in some organs, particularly the liver [76,78,79]. Serum liver enzymes are originally intracellular and are released into bloodstream following cellular damage [45,80–82] since toxicants such as heavy metals increase the permeability of damaged hepatocytes, and consequently enzymes leak out of the liver into the bloodstream and their levels increase. Such an increase leads to anorexia and fatigue [39,40]. In the current study, mean Cd and Pb concentrations in the liver of contaminated–uninfected fishes were distinctly high and led to a significant increase in the levels of serum liver enzymes (ALT, AST, ALP, and GGT), exceeding those in the reference group. Such an increase in the levels of these enzymes in fishes, due to heavy metal pollution, has been reported in numerous studies [45,78,83]. Several studies on the impact of helminth parasites on blood biochemical parameters of their host fishes [46–50] have shown that infection of fishes with helminth parasites such as monogeneans, cestodes and nematodes significantly increases the levels of serum liver enzymes. Controversially, our results revealed that mean Cd and Pb concentrations in the liver of contaminated–infected fishes significantly reduced to approximately half that in uninfected fishes, and, consequently, serum liver enzymes in infected fishes significantly decreased towards their levels in the reference group. These results firmly suggest that infection with acanthocephalans mitigates the impacts of elevated serum liver enzymes on their fish hosts by absorbing considerable amounts of heavy metals from fish intestine.

In fishes, serum glucose level is a sensitive indicator for environmental stress [84–86], and significantly increases (hyperglycemia) after exposure to heavy metal pollution [71,87–89]. Hyperglycemia leads to reduced growth, high feed conversion ratio, and low sustainable growth rate. Toxicity of these metals leads to severe liver damage and stress conditions, which together adversely affect carbohydrate metabolism in fish liver and consequently blood glucose level significantly increases [51,83,90]. In agreement with these observations, our results revealed that mean serum glucose level in contaminated–uninfected fishes was significantly higher than its level in the reference group. Few studies [48,91] have shown that infection with helminth parasites, such as monogeneans, cestodes, and nematodes, significantly increases serum glucose level in infected fishes. On the contrary, our results revealed that mean serum glucose level in contaminated–infected fishes significantly decreased towards its level in reference group and was significantly lower than that in contaminated–uninfected fishes. This firmly suggests that infection with acanthocephala mitigates the impact of elevated blood glucose level on their fish hosts by absorbing considerable amounts of heavy metals from the fish's intestine.

Serum triglyceride level is an essential indicator for the metabolic status of fish and is usually increased (hypertriglyceridemia) in fishes living in polluted environments;

hypertriglyceridemia can cause fatty deposits in the internal organs and blockage of blood vessels [92,93]. Significant changes in its level point to liver dysfunction and consequently inhibition of lipid catabolism [92–95]. Similarly, our results revealed that the mean serum triglyceride level in contaminated–uninfected fishes was significantly higher than its level in the reference group. Generally, studies on the impact of helminth parasites on serum triglycerides level in their host fishes are unavailable. Our results revealed that the mean serum triglyceride level in contaminated–infected fishes significantly decreased towards its level in the reference group and was significantly lower than that in contaminated–uninfected fishes. This strongly suggests that infection with *acanthocephala* mitigates the impact of elevated serum triglycerides level on their fish hosts by absorbing considerable amounts of heavy metals from fish intestine.

In fishes, the serum total protein (STP) level is an essential indicator for liver integrity and general health status [40,41]. STP usually decreases (hypoproteinemia) in fishes inhabiting polluted environments; hypoproteinemia mostly causes slowed growth, muscle loss, and a weakened immune system. The decrease in STP level is mostly due to severe liver damage and stress conditions [44,96]. In agreement with these observations, our results showed that the mean STP level in contaminated–uninfected fishes was significantly lower than its level in the reference group. Several studies [48,91] have shown that infection with helminth parasites, such as monogeneans, cestodes, and nematodes, significantly decreases the STP level in infected fishes. On the contrary, our results revealed that the mean STP level in contaminated–infected fishes moderately increased towards its level in the reference group and was moderately higher than that in contaminated–uninfected fishes. This strongly suggests that infection with *acanthocephala* mitigates the impact of reduced STP level on their fish hosts by absorbing considerable amounts of heavy metals from fish intestine. This suggestion is supported by the negative relationship between metal concentrations (Cd or Pb) in fish liver and worm infrapopulation size in fish intestine, and the positive relationship between STP level and worm infrapopulation size in fish intestine.

Serum albumin level in fishes is of considerable diagnostic value and refers to the integrity of vascular system and liver functions [43], and it usually decreases (hypoalbuminemia) in fishes with severe liver damage due to chronic exposure to pollutants [43,97]. Hypoalbuminemia adversely affects the transport functions through the vascular system in fish [43]. Generally, the degree of deficiency in serum albumin level is directly proportional to the degree of liver damage [98]. These findings are consistent with our results, which revealed that mean serum albumin level in contaminated–uninfected fishes was significantly lower than its level in the reference group. No previous studies are available on the impact of helminth parasites on serum albumin level in their host fishes. Amazingly, our results revealed that mean serum albumin level in contaminated–infected fishes moderately increased towards its level in the reference group and was significantly higher than that in contaminated–uninfected fishes. This strongly suggests that infection with *acanthocephala* mitigates the impact of reduced serum albumin level on their fish hosts by absorbing considerable amounts of heavy metals from fish intestine.

Serum urea level is an essential indicator for the metabolic status of fish and is usually increased (hyperuricemia) in fishes living in polluted environments due to gill and liver diseases [42,99,100] since urea in fish is formed by the liver and excreted mainly by gills as ammonia, and a small amount is excreted by the kidneys as urine [101]. The excess amount of urea (at a high concentration) poisons the cells and adversely affects various cellular processes [42,101]. Our results fully agree with these findings since the mean serum urea level in contaminated–uninfected fishes was significantly higher than its level in the reference group. Many studies [47,49,50] have revealed that helminth parasites, such as monogeneans, cestodes, and nematodes, significantly increase serum urea level in their fish hosts. Controversially, our results revealed that the mean serum urea level in contaminated–infected fishes significantly decreased towards its level in the reference group and was significantly lower than that in contaminated–uninfected fishes. This robustly suggests

that infection with acanthocephala mitigates the impact of elevated serum urea level on their fish hosts by absorbing considerable amounts of heavy metals from fish intestine.

In addition to the above, our results are reinforced by the negative relationships between metal concentration (Cd or Pb) in fish liver, levels of serum liver enzymes, level of serum glucose, level of serum triglycerides, or level of serum urea and worm infrapopulation size in fish intestine, and by the positive relationship between STP level or serum albumin level and worm infrapopulation size in fish intestine.

5. Conclusions

Our results entirely agree with most previous studies in that gutless helminth parasites that absorb their nutrients through their tegument from fish intestine, such as acanthocephalan worms, are a good entry for trace metal pollution, and the current host–parasite system, *Siganus rivulatus*–*Sclerocollum rubrimaris* seems to be very useful for biomonitoring trace metal pollution in the Red Sea. However, the combination of our results suggests that infection with acanthocephalans mitigates the harmful impacts of trace metal pollution on their fish hosts because infection with these worms: (1) significantly decreases the elevated concentrations of both Cd and Pb in fish liver by absorbing and bioaccumulating considerable amounts of these metals from fish intestine; (2) significantly decreases the elevated levels of liver enzymes, glucose, triglycerides, and urea in fish blood serum towards their levels in the reference group; and (3) moderately increases the declined levels of total protein and albumin in fish blood serum towards their levels in the reference group. Finally, we can say that “acanthocephalan worms are not absolutely harmful to their fish hosts, but sometimes have beneficial impacts on these hosts”.

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