



Role of Parasitic Helminths in Bioremediating Some Heavy Metal Accumulation in the Tissues of *Lethrinus mahsena*

Amaal Hassan^{1,2,*}, Samia Moharram³, Hanan El Helaly¹

¹ Biology Department, Science College, King Abdulaziz University, Jeddah, Saudi Arabia.

² Zoology Department, Faculty of Science, Sohag University, Sohag, Egypt.

³ National Institute of Oceanography and Fisheries, Alexandria, Egypt.

* Corresponding Author: Tel.: +96,6502679429;
E-mail: ahasanmo@gmail.com

Received 19 May 2017
Accepted 24 July 2017

Abstract

Fish take up metals from the aquatic ecosystem and accumulate it in their muscle tissues and organs. The aim of the present study was to determine the role of intestinal helminths in bioremediating the accumulation of some heavy metals in the muscle tissues of the emperor fish, *Lethrinus mahsena*. A total of 130 fish were collected randomly from Raas Mehzen in the Red Sea, Jeddah coast, during the period of November 2015 to May 2016. The percent of total infection in *L. mahsena* fish was found to be 62%. Four species of Trematoda, one cestode larva and three species of Nematoda were recorded from the small intestine. Element concentrations in the infected and non-infected fish and parasites were analyzed using flame atomic absorption spectrometry. Mean concentration of individual heavy metals in trematode parasites was in the order of As > Fe > Zn > Pb > Cu > Cd. For cestodes, it was As > Fe > Zn > Pb > Cu > Cd. While in nematodes, it was in the order of As > Fe > Zn > Pb > Cd > Cu. Most heavy metals exhibit a significant decrease in fish tissues infected with helminths. Bioaccumulation factors indicated much higher concentrating capacity of cestode parasites than fish organs. Thereby, cestode and nematode infections in marine fish could be considered to be a biological indicator of heavy metal pollution and at the same time could also minimize the bioaccumulation of heavy metals in fish tissues.

Keywords: Heavy metals, tissues, helminths, bioaccumulation, *Lethrinus mahsena*.

Introduction

Heavy metals are considered to be the most important form of pollutants in the aquatic environment due to their toxicity and tendency to be accumulated by marine organisms. Jeddah Coast is believed to be contaminated with heavy metals largely from the growing number of industries, then shipping and human wastes. Numerous industries are located in and around Jeddah coast due to its strategic location as one of the shipping industry and urbanization centers of Saudi Arabia; but the same time these industries are source of toxic chemicals and effluents (Ali, Elazein, & Alian, 2011a). Many species of marine benthic fish are shown to reflect environmental status. However, seasonal changes may change heavy metal concentrations in fish tissues, thereby affecting the accuracy of the advisories related to fish consumption information and assessment of risk (Conte *et al.*, 2015). Contaminants accumulated in various fish provide an estimate of integrated metal exposure. The muscle (flesh) is the most commonly chosen tissue because of the implications it carries for human consumption and

health risk. Fish could therefore be possibly used as indicators in areas affected by human activities to describe the state of the environment (Akinsanya, Otubanjo, & Ibidapo, 2007; Shreadah, Abdel Fattah, & Fahmy, 2015).

Heavy metals, such as iron (Fe), copper (Cu) and zinc (Zn), are important for fish metabolism, while cadmium (Cd), lead (Pb), mercury (Hg) and others have unknown functions in biological systems (Velusamy, Satheesh, Ram, & Chinnadurai, 2014). Heavy metals enter fish by direct absorption from water through their gills and skin or by ingestion of contaminated food. The metals then enter the fish's blood stream and ultimately gets accumulated into their tissues, mainly liver, where they are bio-transformed and excreted or taken up through the food chain to consumers (Mziray & Kimirei, 2016).

Parasites can be used either as effect indicators or as accumulation indicators because of the variety of ways in which they respond to anthropogenic pollution (Sures, 2004; Uçkun, 2017). Accumulation indicators are organisms which can concentrate certain substances in their tissues to levels significantly higher than those in the ambient

environment. They provide valuable information about the chemical state of their habitats (Beeby, 2001). Many types of pollutants are parasitized and under natural environmental conditions, most fish parasites are believed to cause little or no harm (Azmat, Fayyaz, Kazi, Mahmood, & Uddin, 2008). Using parasites as bioindicators of heavy metal accumulation is a promising approach due to their capacity to bio-concentrate trace elements.

Materials and Methods

The fish collection site is Raas Mehsen. It is located at Al-Lith beach, about 170 km south of Jeddah city, with global positioning system (GPS) reading of 28° 37' 20.15 N and 29° 33' 40.27 E. This site receives industrial effluents discharged from the nearby factories. It represents the main coast of Jeddah city and receives continuous untreated domestic waste water from the nearby city (Figure 1).

A total of 130 fish were collected randomly from Raas Mehsen at the Red Sea, Jeddah coast, Saudi Arabia, during the period from November 2015 to May 2016. Fresh fish were dissected and examined microscopically for endo-parasites. Helminths were collected from infected organs via dropper and washed several times in petri dishes containing saline solution to remove debris and mucus. Parasites were identified according to their morphological features depending on taxonomic keys.

Heavy Metals Analysis

Heavy metals in water samples were extracted with conc. HCl and preserved in a refrigerator till analysis for As, Cd, Cu, Fe, Pb and Zn concentrations (Ali, Elazein, & Alian, 2011b). One gram of fish muscles, liver, kidney and intestine were placed in crucibles. These crucibles were left in hot oven at

80°C overnight. Then the tissues were crushed using mortar and pestle, weighed, and transferred into 100 ml flasks. About 10 ml of concentrated nitric acid was added into each flask, and then boiled on a hot plate until the nitric acid dries up. The final step involved adding 25 ml of distilled water into the flasks followed by the addition of one ml of HCl. The solution was then analyzed by Flame Atomic Absorption Spectrometry (Biology Department, Science College, King Abdul Aziz University, Saudi Arabia). Flame Atomic Absorption Spectrometry (FAAS) was performed using Varian 5-AAS analytic Jena Spectrometer to determine As, Cd, Cu, Fe, Pb and Zn concentrations. The flame wave length and sample aspiration rate were optimized according to the manufacturer's recommendations, and four aqueous standards having analytical concentration within the linear response range of the instrument and containing the same concentration of nitric acid as the sample were used for calibration. Each sample, standard and blank, was analyzed using three 10-s integration. The reagent blank, was prepared and its value was subtracted to give the final concentration in mg/g (Hassan, Al-Zanbagi, & Al-Nabati, 2016).

Statistical Analysis

The data were analyzed using t-test analysis to compare the bioremediation capacity between infected and non-infected fish. The differences in heavy metal concentrations between non-infected and infected tissues, and parasites species were tested separately. For comparing two independent groups, Independent sample t-test was used. The mean metal concentration $\times 1/\text{dry-weight}$ for sample was used to calculate the capacity of metal concentration mg/L and then the equation (mineral concentration capacity $\times 25$)1000 was used for calculating the capacity of mineral concentration mg/g (25 volume of

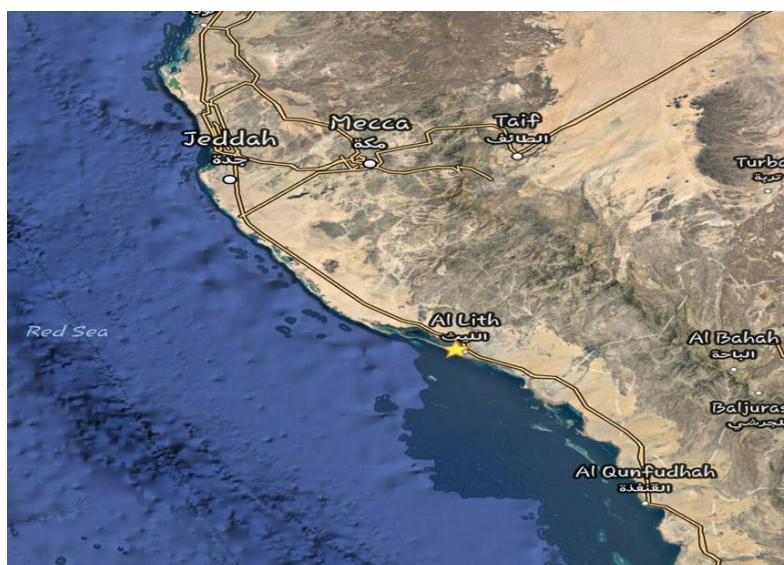


Figure 1. Map of the studied site (Google map).

samples). The bioaccumulation factors (BF) were calculated according to Sures, Siddall, and Taraschewski (1999), as the ratio of the element concentration in the parasites to that in different host tissues i.e. $BF = (C [\text{parasite}] / (C [\text{host tissue}]])$. All statistical analysis was performed using SPSS software program (version 19).

Results

The total percentage of *Lethrinus mahsena* fish infected with helminths from Raas Mahsen site was found to be 62%. The infection with trematodes was the most prevalent in *L. mahsena* (44%) followed by nematodes 28% while the lowest infection percentage was cestodes (22%). Eight helminth species were collected from the emperor fish intestine. Four of them were found belonging to class: Trematoda; *Harmacreadium khalili* constituting about 12.90%, *Hamacreadium mutabile* was 22.58%, *Hamacreadium cribbi* was 9.68% and *Pseudoplagioporus lethrini* was 3.22%, then one cestode; *Floriceps* was about 25.0% and then among the three nematode species; *Terranova* sp. was 6.45%, *Cucullanus maldivensis* was 6.45% and *Ascarophisnema tridentatum* was 6.45%.

Then the emperor fish, *L. mahsena* muscle tissues, liver, kidney and intestine and their intestinal parasites (trematodes, cestodes and nematodes) as well as sea water were analyzed for the presence of six heavy metals: arsenic (As), cadmium (Cd), copper

(Cu), iron (Fe), lead (Pb) and zinc (Zn) using flame atomic absorption spectrometry. Heavy metal concentrations (mg/L) in water were found out to be in the following order: As 56.76, Zn 14.12, Pb 0.889, Fe 0.123, Cd 0.082 and Cu 0.078.

In the non- infected fish, muscles were the main site for Cu accumulation, while the highest quantity of Pb was in the liver followed by muscle tissues. Whereas, As and Zn were mainly accumulated in the kidney of the emperor fish. On the other hand, intestine was the main site for Cd accumulation. Then kidney was found to be the main site of Fe accumulation (Table 1).

Shown in table 1 are the alterations of metal uptake and accumulation in fish tissues and organs due to intestinal parasites. In the muscle tissues, As and Fe were lower in the fish muscles infected with cestode larvae whereas they were higher in the tissues of fish infected with intestinal trematodes and nematodes. There was also a significant decrease in Cd and Cu. On the other hand, Pb and Zn significantly increased in cestode and nematode infected fish but insignificantly in trematode infected fish muscles.

In the fish liver, Cd decreased significantly in cestode infected fish but insignificantly in trematode and nematode infections. Arsenic and Fe insignificantly decreased in cestode infections whereas, Pb and Zn insignificantly decreased in the case of nematodes. Copper decreased significantly in the case of cestode infected fish and insignificantly in

Table 1. Heavy metals concentration (mg/g) in *Lethrinus mahsena* tissues infected with different helminth parasites

| Tissues | Control | Trematodes | P value | Cestodes | P value | Nematodes | P value |
|-----------|----------------|----------------|---------|---------------|---------|----------------|---------|
| | | | | As | | | |
| Muscle | 386.03±50.97 | 902.04±28.20 | 0.000* | 373.25±20.45 | 0.129 | 1025.45±217.37 | 0.001* |
| Liver | 571.32±93.68 | 1234.96±415.06 | 0.005* | 423.80±48.50 | 0.217 | 941.92±175.01 | 0.542 |
| Kidney | 1218.62±275.92 | 687.02±135.31 | 0.036* | 604.08±507.92 | 0.437 | 1234.67±978.40 | 0.149 |
| Intestine | 372.37±82.05 | 541.46±56.00 | 0.137 | 686.80±60.00 | 0.269 | 681.51±300.20 | 0.119 |
| | | | | Cd | | | |
| Muscle | 0.017±0.008 | 0.005±0.002 | 0.000* | 0.005±0.004 | 0.03* | 0.003±0.001 | 0.008* |
| Liver | 0.165±0.022 | 0.159±0.048 | 0.749 | 0.019±0.002 | 0.01* | 0.128±0.043 | 0.946 |
| Kidney | 0.042±0.008 | 0.047±0.039 | 0.041* | 0.007±0.002 | 0.045* | 0.024±0.016 | 0.647 |
| Intestine | 1.585±1.554 | 0.009±0.002 | 0.028* | 0.006±0.003 | 0.032* | 0.021±0.012 | 0.024* |
| | | | | Cu | | | |
| Muscle | 0.096±0.046 | 0.027±0.013 | 0.057* | 0.005±0.001 | 0.020* | 0.023±0.010 | 0.026* |
| Liver | 0.152±0.016 | 0.155±0.045 | 0.223 | 0.042±0.002 | 0.020* | 0.114±0.018 | 0.431 |
| Kidney | 0.047±0.011 | 0.018±0.005 | 0.011* | 0.005±0.000 | 0.048* | 0.035±0.024 | 0.968 |
| Intestine | 0.106±0.043 | 0.033±0.007 | 0.021* | 0.016±0.005 | 0.05* | 0.067±0.032 | 0.029* |
| | | | | Fe | | | |
| Muscle | 2.949±0.954 | 6.985±5.029 | 0.018* | 0.191±0.071 | 0.019* | 4.353±2.558 | 0.248 |
| Liver | 6.208±0.959 | 9.224±2.624 | 0.173 | 1.420±1.080 | 0.213 | 11.485±6.793 | 0.009* |
| Kidney | 15.483±5.432 | 3.696±0.814 | 0.017* | 1.760±1.197 | 0.041* | 4.918±2.762 | 0.037* |
| Intestine | 14.429±7.560 | 2.756±0.952 | 0.013* | 0.248±0.144 | 0.030* | 3.837±1.494 | 0.012* |
| | | | | Pb | | | |
| Muscle | 0.076±0.009 | 0.103±0.014 | 0.210 | 0.128±0.001 | 0.001* | 0.105±0.009 | 0.011* |
| Liver | 0.098±0.016 | 0.102±0.019 | 0.938 | 0.120±0.006 | 0.469 | 0.084±0.018 | 0.928 |
| Kidney | 0.318±0.042 | 0.160±0.047 | 0.221 | 0.249±0.147 | 0.945 | 0.174±0.057 | 0.359 |
| Intestine | 0.218±0.062 | 0.068±0.018 | 0.049* | 0.110±0.024 | 0.019* | 0.182±0.042 | 0.012* |
| | | | | Zn | | | |
| Muscle | 0.948±0.326 | 1.436±0.876 | 0.441 | 3.014±2.596 | 0.032* | 3.640±2.527 | 0.002* |
| Liver | 3.607±0.323 | 4.675±0.788 | 0.469 | 4.818±0.890 | 0.825 | 3.415±0.550 | 0.955 |
| Kidney | 9.698±1.849 | 2.787±1.360 | 0.126 | 4.218±3.027 | 0.407 | 2.672±0.640 | 0.034* |
| Intestine | 3.180±1.298 | 2.272±1.590 | 0.813 | 4.877±3.421 | 0.911 | 2.051±0.575 | 0.352 |

nematode infected fish whereas; there was an insignificant increase in nematode infections. Arsenic was insignificantly high in nematode but it was significant in trematode infections. Then, Cu in trematode infection, Fe in trematode and nematode, and Pb and Zn in trematode and cestode infections were insignificantly high compared with non-infected fish.

Kidney of infected fish had lower concentrations of Cu, Fe, Pb and Zn; whereas, Cd was significantly low in the case of cestodes, but insignificantly low in nematode and it was also significantly high in the trematode infected fish. Then, As was low in trematode and cestode infections but insignificantly high in infected fish kidney.

In the intestine of infected fish, there was a significant decrease in the concentrations of Cd, Cu, Fe and Pb but an insignificant decrease in Zn in the case of trematode and nematode infected fish as compared to the control. While, it was insignificantly high in cestode infected fish. But, As was found to be insignificantly increased in all the helminth infections.

The metal concentrations in trematode parasites was in the order of As > Fe > Zn > Pb > Cu > Cd. For cestodes, it was As > Fe > Zn > Pb > Cu > Cd. While in nematode parasites, metal concentrations were in the order of As > Fe > Zn > Pb > Cd > Cu (Table 2, Figure 2).

Bioaccumulation Factors (BFs)

The differences among most concentrations of heavy metals accumulated in the muscles, liver, kidney and intestine of emperor fish and those in parasites were found to be statistically significant

(Table 3). This means that, accumulation capacity of some heavy metals (Fe, Cd, Cu and Pb) was found to be significantly higher in some helminths than in emperor fish tissues. The highest Bioaccumulation factor (BF= C in parasite/C in tissues) was obtained for Fe (BF= 43.18) which presented accumulation ratio between parasites and fish organs higher than other elements. Iron absorption in cestodes was 226 times higher than fish muscles, Cadmium BFs in nematodes was similarly high (BFs=16.08, 1.76 and 1.57, respectively), which was approximately 331, 83 and 65 times higher than Cd concentration detected in fish muscles, intestine and kidney, respectively. Cadmium BF in cestodes was 1.227 which is about 64 times higher than that detected in fish liver, followed by trematodes (BF= 0.283 and 0.15), which is about 56 and 16 times higher than that detected in fish muscles and intestine, respectively. The highest BF of copper was detected in cestodes (BF= 9) followed by trematodes (BF= 0.199) and nematodes (BF= 0.101) which is about 1800, 7 and 4 times higher compared with Cu concentration detected in fish muscles. Bioaccumulation factor of Cu in trematodes was 0.16 which is about 5 times higher than that detected in fish intestine. Lead BFs in cestodes, nematodes and trematodes were 0.62, 0.4 and 0.276, respectively and this is about 4, 3, 2 times higher compared with its concentration detected in fish muscles. Lead BFs in cestodes, nematodes and trematodes were found to be 0.66, 0.5 and 0.28, respectively, which is about 5, 6 and 3 times higher than Pb concentration in fish liver. Bioaccumulation factor for lead in trematodes was 0.416, which is about 6 times higher than that found in the fish intestine.

Table 2. Metal concentrations (mg/g) in helminth parasites collected from the emperor fish, *Lethrinus mahsena*

| Minerals | Trematodes | Cestodes | Nematodes |
|----------|--------------|---------------|---------------|
| As | 1423.2±576.8 | 4654.4±1345.6 | 291.92±108.08 |
| Cd | 0.021±0.009 | 0.156±0.044 | 0.077±0.017 |
| Cu | 0.092±0.038 | 0.312±0.088 | 0.029±0.011 |
| Fe | 28.467±11.53 | 61.58±18.42 | 14.59±5.415 |
| Pb | 0.428±0.172 | 0.546±0.154 | 0.508±0.192 |
| Zn | 1.431±0.569 | 4.686±1.314 | 0.581±0.219 |

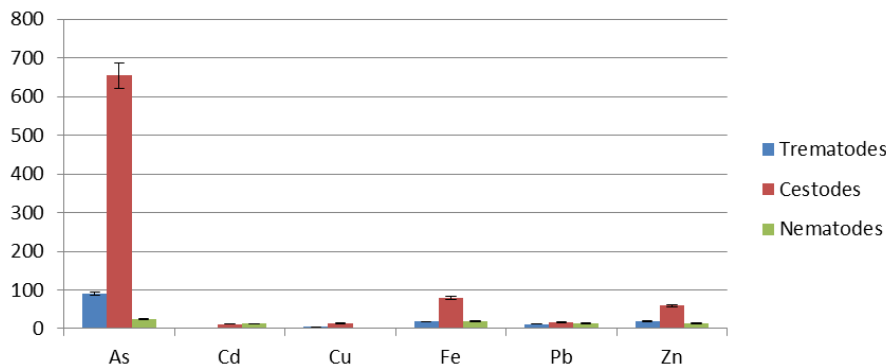


Figure 2. Metals concentrations (mg/g) in parasites tissues.

Table 3. Bioaccumulation factors (BFs) calculated for the analyzed metals in parasites and respective emperor fish tissues, Significant *P ≤ 0.05, **P ≤ 0.01

| Tissues | Parasites | Minerals | | | | | |
|-----------|------------|----------|---------|--------|--------|---------|-------|
| | | As | Cd | Cu | Fe | Pb | Zn |
| Muscle | Trematodes | 0.099 | 0.283** | 0.199* | 0.258 | 0.276** | 0.068 |
| | Cestodes | 1.753 | 4.54 | 9* | 43.18* | 0.62* | 0.23 |
| | Nematodes | 0.025 | 16.08* | 0.101* | 0.29 | 0.40** | 0.01 |
| Liver | Trematodes | 0.073 | 0.008 | 0.034 | 0.195 | 0.28** | 0.021 |
| | Cestodes | 1.544 | 1.227* | 1.08 | 5.81 | 0.66** | 0.142 |
| | Nematodes | 0.027 | 0.287 | 0.020 | 0.109 | 0.5** | 0.014 |
| Kidney | Trematodes | 0.131 | 0.029 | 0.297 | 0.487 | 0.177 | 0.035 |
| | Cestodes | 1.083 | 3.492 | 9 | 4.687 | 0.319 | 0.163 |
| | Nematodes | 0.020 | 1.57* | 0.065 | 0.255 | 0.240 | 0.018 |
| Intestine | Trematodes | 0.17 | 0.15* | 0.16* | 0.653 | 0.416* | 0.043 |
| | Cestodes | 0.95 | 4.13 | 2.81 | 33.32* | 0.72* | 0.14 |
| | Nematodes | 0.037 | 1.76* | 0.03 | 0.33 | 0.23 | 0.023 |

Discussion

Certain organisms are able to provide information about the chemical state of the environment through their presence or absence. Some organisms are less affected by toxic substances, and also show an ability to concentrate environmental pollutants inside their tissues (Rosenberg & Resh, 1993). In this regard, even though parasites do not accumulate organic pollutants, but they are able to alter the uptake of chemicals of their hosts, including metals (Evans, Irwin, & Fitzpatrick, 2001). Parasites accumulate greater concentrations of toxic metals in their bodies, thereby acting as metal sinks for the fish host and thereby aiding in their survival in the presence of toxins (Azmat *et al.*, 2008; Eissa, Gehan, Wafeek, & Nashwa, 2012; Sures, 2007). Therefore, it is necessary from a public health viewpoint to determine the heavy metal concentrations in fish captured for human consumption (Khan & Thulin, 1991).

In the present study, six heavy metal concentrations (mg/L) in the water of Raas Mahsen site at Jeddah coast (As, Cd, Cu, Fe, Pb and Zn) were analyzed. The high levels of some heavy metals in water of Jeddah port could be attributed to the shipping industry and agricultural discharge as well as from spill of leaded petrol from fishing boats and dust which holds a huge amount of lead from the combustion of petrol in automobile cars (Banat, Nigam, Singh, Marchant, & McHale, 1998.)

The present study found that the concentrations of Cd, Cu, Fe, Pb and Zn in the tissues of non-infected fish, *Lethrinus mahsena* were found to be below the Saudi Arabian Standards Organization (SASO) limits except for Arsenic. The highest Cd concentration was detected in the intestine and this is in conformity with the study of Ali *et al.* (2011a). Cadmium when detected occurred at low levels and which was within SASO limits in the muscle tissues of fish. High mean concentrations of Cu in the present study were found in the muscle tissues which are

contradictory with the study of Ali *et al.* (2011b) who found highest concentration of the metal in the liver of *Lethrinus nebulosus*. Zinc concentration was higher when compared with Cd, Cu and Pb. It occurred in kidney, intestine and liver, respectively. Similar results of Zn concentration in muscles of several marine fish species has also been reported by Abul-Naja (1996). The highest concentration of Pb was found in the kidney and intestine of the emperor fish, *L. mahsena*. Moore and Rammamoorth (1984) recorded little accumulation of Pb in the muscles of marine and freshwater fish species. Low concentration of Pb is found in the muscles of marine fish from coastal areas in England and Wales (<1.0 µg/g wet weight); then in west Malaysia (<0.5 mg/kg wet weight) and Gulf of Aqaba (0.8- 2.6 µg/g wet weight) (Wahbeh & Mahasneh, 1987). In fishes from the Red Sea Pb ranged between 0.01-0.66 µg/g in the muscles, 0.1-2.4µg/g in the liver and <0.05-0.14 µg/g in the gonads. The present data indicate that the fish *Lethrinus mahsena* in the Red Sea contain higher levels of Pb compared with other fishes in the world.

Generally, worms interfere with the host's absorption, metabolism, uptake and regulation of the mineral elements (Poulin, 1998; Barber, Hoare, & Krause, 2000; Samuel & George, 2000; Sures, 2003; Kelly & Janz, 2008; Minguéz, Meyer, Molloy, & Giambérini, 2009). Penetration and nourishment on the host tissues occur by proteinases such as metalloproteinases secreted by adult nematodes, adult trematodes, cercariae and plerocercoids (Dzik, 2005). Accordingly, the presence of helminth can alter the metabolic and physiological function of fish (Navratil, Palikova, & Klement, 1999; Baruš, Tenora, Proke, & PeAz, 2001; Azmat *et al.*, 2008). Parasites and their host compete for several elements such as Cu, Fe and Zn (Gabrashanska & Nedeva, 1996; Sures, 2002; Oyoo-Okoth, Admiraal, Osano, Hoitinga, & Kraak., 2010; Oyoo-Okoth, *et al.*, 2012). Parasites affect the host's immunological responses by secreting hormones, neuropeptides, or cytokine like molecules or factors that directly alter the host's hormone levels

(Beckage, 1993; Jobling & Tyler, 2003). Parasites do cause endocrine disruption and such is the case with cestode, *Ligula intestinalis* which interfere with the hypothalamus pituitary–gonadal axis of its host (Williams, Penlington, King, Hoole, & Arme, 1998) and can thus impede the process of element uptake (Oyoo-Okoth *et al.*, 2010). Further, loss of blood in the host during the parasite's life cycle affects the concentration of several microelements in its tissues (Schulz & Gvozdev, 1972; Brand, 1973).

Intestinal parasites are the ones which get more metals as compared to parasites inhabiting other parts of the fish body (Nachev, Schertzinger, & Sures, 2013). Oyoo-Okoth *et al.* (2012) suggested that cestodes interfere with the metal detoxification role of metallo-proteins in the host where they absorb metal binding proteins along with numerous lipids and proteins required for metabolic needs; and explain the reason for the increase of some metals in infected fish compared to the non-infected ones. The amount of several microelements in host tissues may be affected by blood loss during the parasite's life cycle with the utmost impact being on Fe and Cu which are involved in the blood production process (Schulz & Gvozdev, 1972; Brand, 1973; Baruš, Tenora, Krácmár, & Dvoraček, 1999). Iron is a fundamental part of hemoglobin and is bound to proteins such as ferritin. Forstner and Wittmann (1979) showed that Pb affect membrane permeability of fish kidney, liver and brain cells whereby it inhibits the biosynthesis of haem thereby causing functional disruption or breakdown of these tissues. The increase of element concentration in fish muscles during infection may be due to compensatory reaction of the host (Samuel & George, 2000). Hassan, Akinsanya, and Olakolu (2013) found higher concentration of metals in the intestinal wall of the fish host when infected by the nematodes, *Contracaecum* sp. and *Raphidascaris* sp. suggesting a low efficiency of these nematodes in accumulating of metals, and this may explain the increase in some metals in infected fish.

Parasitic helminths may play an important role because of their capability of accumulating heavy metals in their bodies. Some helminths are suggested to have a high efficiency of bioaccumulation of metals; whereas, others have a low efficiency in absorption of metals from the emperor fish, *L. mahsena*. This phenomenon of heavy metal bio-concentration also appears to be closely linked to intestinal location of the parasites. Certain parasites can accumulate heavy metals at concentrations higher than their host tissues and organs (Sures, Taraschewski, & Rydlo, 1997; Zaghoul, Abd El-Monem, Fayed, & Mohamed, 2001). In some examples, infected fish have been shown to accumulate both greater (Pascoe & Cram, 1977) and lesser (Sures *et al.*, 1999; Sures, 2003) number of contaminants than non-parasitized fish. The most detailed studies in marine systems were performed with cestodes and nematodes. Most data available for

cestodes are concentrated on Pb and Cd. Tetraphyllidean tapeworms showed the highest accumulation capacity and metals levels in the tapeworms were found to be 460 times higher than the host muscle tissues (Malek, Haseli, Mobedi, Ganjali, & Mackenzie, 2007). Concerning nematodes, *Ascaris* and *Echinocephalus* exhibit better accumulation features due to the fact that they grow and feed on the fish host which is an important way of pollutant uptake.

Since parasites accumulate toxic metals in their body thereby they act as metal sinks for its fish host and help them survive in spite of the presence of toxins (Sures, 2007; Azmat *et al.*, 2008; Eissa *et al.*, 2012). The ratio between metal level in the parasites and their host tissues (Bioaccumulation factor, BF) provide evidence about the duration of the exposure, as the accumulation occurs more rapidly in the parasite. Accordingly, high metal levels in the host muscle and a correspondingly lower bioaccumulation factor indicate longer exposure (Sures & Roy, 2001). In the present study, the bioaccumulation factors for most heavy metals confirmed the high accumulation capacity of parasites compared to fish tissues. Bioaccumulation factor in four metals, i. e. Fe, Cd, Cu and Pb was higher in intestinal helminths than the fish tissues. The highest BF was obtained for Fe, which presented accumulation ratios between parasites and fish organs (muscles and intestine). The maximum BF of iron was stated for cestodes and muscles of emperor fish (BF=43.18). Concentrations of Cd in nematodes similarly attained much higher levels compared to fish organs (muscle, intestine and kidney) followed by trematodes when compared with fish muscles and intestine, and then cestodes with the liver. There was a significantly higher accumulation capacity of Cu in cestodes, trematodes and nematodes in relation to fish muscles and trematodes in relation to fish intestine. Lead concentration was higher in cestodes, followed by nematodes and trematodes, respectively compared to liver and muscle tissues and trematodes in relation to the intestine. Parasites such as cestodes impede the metal detoxification or regulatory mechanisms of metals in fish (Oyoo-Okoth *et al.*, 2012). Interruption of the hepatic intestinal cycle was observed due to intestinal parasites such as cestodes (Sures *et al.*, 1999; Torres, Eira, Miquel, & Feliu, 2012). The digestive tract is absent in cestodes, therefore they are able to concentrate metals to a higher degree than the host tissues (Thielen, Zimmermann, Baska, Taraschewski, & Sures, 2004). Worms are unable to synthesize their own cholesterol and fatty acids; so they take bile from host intestine which in turn reduces their ability to absorb metals (Sures, 2003; Malek *et al.*, 2007). Cestodes use the bile salts for their egg formation and hatching (Sures *et al.*, 1999; Retief, Avenant-Oldewage, & Du Preez, 2009). This could possibly explain the high concentration of metals in these parasites.

Adult nematode infection in marine fish minimized the bioaccumulation of heavy metals in fish tissues and organs; and therefore was considered as a biological indicator of heavy metal pollution (Eissa *et al.*, 2012). Among the nematodes, adult philometrids and both adults and larvae of *Anisakis* concentrated metals efficiently whereas other species displayed no concentrating ability at all or are able to concentrate few metals only (Tenora, Baruš, & Prokes, 2002; Pascual & Abollo, 2003; Baruš, Jarkovský, & Prokeš, 2007; de Buron, *et al.*, 2009). The larval nematodes through their different uptake routes and because of the need to grow fast during their development, therefore tends to adsorb essential elements such as (Cu, Fe, and Zn) through their body (Nachev *et al.*, 2013; Hassan *et al.*, 2016).

In conclusion, intestinal cestodes and nematodes caused a significant decrease in Cu, Fe, and Zn and an insignificant decrease in Cd and Pb concentrations in the infected fish tissues and organs. This indicates that intestinal helminths can reduce the level of metals in the *L. mahsena* tissues and organs; and this can be advantageous for the infected hosts, allowing them to tolerate much higher concentrations of certain metals. The present results also confirmed that nematodes, cestodes and trematodes seem to be good indicators of environmental conditions. This argument could be supported from the results of the present study which demonstrated the ability of these parasites to accumulate iron, cadmium, copper and lead. The present results also show the usefulness of the marine endoparasites for the possible application as early warning indicators of heavy metals pollution.

But to ultimately use parasites as indicators, needs more information and understanding about the basic physiological effect of parasites on their hosts at the time of infection, development and reproduction. Hence, this is a field of growing importance, where parasitologists can try to combine their knowledge with toxicologists and eco-toxicologists. Then only the phenomenon of conspicuous metal accumulation by parasites might be applied to environmental monitoring. Furthermore, marine ecologists could also consider parasitism in environmental studies, not only as a health factor of the hosts, but also as a tool for providing additional information about the ecological state of marine ecosystems. This opens up the possibility of how environmental science and parasitology might profit from each other in the near future.

References

- Abul-Naja, W.M. (1996). Comparative study of trace metals accumulated in the muscle tissues of the most marketable seafood in Alexandria waters. *International Journal Environmental Health Research*, 6(4), 289-300.
- Ali, A.A., Elazein, E.M., & Alian, M.A. (2011a). Determination of Heavy Metals in four common Fish, Water and Sediment Collected from Red Sea at Jeddah Isalmic Port Coast. *Journal of Applied Environmental and Biological Sciences*, 1(10), 453-459.
- Ali, A.A., Elazein, E.M., & Alian, M.A. (2011b). Investigation of Heavy Metals Pollution in Water, Sediment and Fish at Red Sea-Jeddah Coast-KSA at two different locations. *Journal of Applied Environmental Biological Sciences*, 1, 630-637.
- Akinsanya, B., Otubanjo, O.A., & Ibidapo, C.A. (2007). Helminth Bioload of *Chrysichthys nigrodigitatus* (Lacepede 1802) from Lekki Lagoon Lagos, Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences*, 7, 83-87.
- Azmat, R., Fayyaz, S., Kazi, N., Mahmood, S. J., & Uddin, F. (2008). Natural bioremediation of heavy metals through nematode parasite of fish. *Biotechnology*, 7(1), 139-143. doi: 10.3923/biotech.2008.139.143.
- Banat, I.M., Nigam, P., Singh, D., Marchant, R., & McHale, P. (1998). Review: Ethanol production at elevated temperatures and alcohol concentrations: Part I—yeasts in general. *World Journal of Microbiology and Biotechnology*, 14(6), 809-821. <https://doi.org/10.1023/A:1008802704374>.
- Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behavior: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, 10(2), 131-165. <https://doi.org/10.1023/A:1016658224470>.
- Baruš, V., Tenora, F., Krácmár, S., & Dvoráček, J. (1999). Contents of several inorganic substances in European eel infected and uninfected by *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms*, 37(2), 135-137. doi: 10.3354/dao 037135.
- Baruš, V., Tenora, F., Proke, M., & PeAz, M. (2001). Heavy metals in parasites host system: Tapeworm vs. fish in Czech. *Ochrana zdravyb* (Health protection of fish). Vodaany, pp: 20-28. doi:10.2754/avb201281030313.
- Baruš, V., Jarkovský, J., & Prokeš, M., (2007). *Philometra ovata* (Nematoda: Philometroidea): a potential sentinel species of heavy metal accumulation. *Parasitology Research*, 100(5), 929-933. doi 10.1007/s00436-006-0384-8.
- Beckage, N. E. (1993). Endocrine and neuro-endocrine host-parasite relationships. *Receptor*, 3(3), 233-245.
- Beeby, A. (2001). What do sentinels stand for? *Environmental Pollution*, 112(2), 285-298. [https://doi.org/10.1016/S0269-7491\(00\)00038-5](https://doi.org/10.1016/S0269-7491(00)00038-5).
- Brand, T. (1973). *Biochemistry of parasites*, 2nd edn. New York, Academic Press. 512 pp.
- Conte, F., Copat, C., Longo, S., Conti, G.O., Grasso, A., Arena, G., Brundo, M.V., & Ferrante, M. (2015). "First data on trace elements in *Haliotis tuberculata* (Linnaeus, 1758) from southern Italy: Safety issues." *Food and Chemical Toxicology*, 81, 143-150. doi: 10.1016/j.fct.2015.04.020.
- De Buron, I., James, E., Riggs-Gelasco, P., Ringwood, A.H., Rolando, E., & Richardson, D. (2009). Overview of the status of heavy metal accumulation by helminths with a note on the use of in vitro culture of adult acanthocephalans to study the mechanisms of bioaccumulation. *Neotropical Helminthology*, 3(2), 101-110.
- Dzik, J.M. (2005). Molecules released by helminth parasites involved in host colonization. *Acta Biochimica Polonica*, 53(1), 33-64.

- Eissa, I.A.M., Gehan, I.S., Wafeek, M., & Nashwa, A.S. (2012). Bioremediation for heavy metals in some Red Sea fishes in Suez, Egypt. *SCVMJ*, XVII, (2), 341-356.
- Evans, D.W., Irwin, S.W.B., & Fitzpatrick, S. (2001). The effect of digenean (Platyhelminthes) infections on heavy metal concentrations in *Littorina littorea*. *Journal of the Marine Biological Association of the UK*, 81 (02), 349-350. <https://doi.org/10.1017/S0025315401003873>.
- Forstner, U., & Wittmann, G.T. (1979). Metal pollution in the aquatic environment. Springer-Verlag, New York, 486pp. doi: 10.1002/iroh.19810660232.
- Gabrashanska, M., & Nedeva, I. (1996). Content of heavy metals in the system fish-cestodes. *Parassitologia*, 38, 58.
- Hassan, A.A., Akinsanya, B., & Olakolu, F.C. (2013). Heavy metal concentrations in nematode parasites of *Clarias gariepinus* and *Synodontis clarias* from Lekki Lagoon, Lagos, Nigeria. *Journal of Marine Science: Research and Development*, 14, 1 – 8.
- Hassan, A., Al-Zanbagi, N., & Al-Nabati, E. (2016). Impact of nematode helminths on metal concentrations in the muscles of koshar fish, *Epinephelus summana*, in Jeddah, Saudi Arabia. *The Journal of Basic & Applied Zoology*, 74, 56-61. <https://doi.org/10.1016/j.jobaz.2016.09.001>.
- Jobling, S., & Tyler, C.R. (2003). Endocrine disruption, parasites and pollutants in wild freshwater fish. *Parasitology*, 126(07), S103-S108. <https://doi.org/10.1017/S0031182003003652>.
- Kelly, J.M., & Janz, D. (2008). Altered energetics and parasitism in juvenile northern pike (*Esox lucius*) inhabiting metal-mining contaminated lakes. *Ecotoxicology and Environmental Safety*, 70, 357–369. <https://doi.org/10.1016/j.ecoenv.2008.01.022>.
- Khan, R., & Thulin, J. (1991). Influence of pollution on parasites of aquatic animals. *Advances in Parasitology*, 30, 201-238. [https://doi.org/10.1016/S0065-308X\(08\)60309-7](https://doi.org/10.1016/S0065-308X(08)60309-7).
- Malek, M., Haseli, M., Mobedi, I., Ganjali, M.R., & Mackenzie, K. (2007). Parasites as heavy metal bioindicators in the shark *Carcharhinus dussumieri* from the Persian Gulf. *Parasitology*, 134(07), 1053-1056. doi: 10.1017/S0031182007002508.
- Minguez, L., Meyer, A., Molloy, D.P., & Giambérini, L. (2009). Interactions between parasitism and biological responses in zebra mussels (*Dreissena polymorpha*): importance in ecotoxicological studies. *Environmental Research*, 109(7), 843-850. doi: 10.1016/j.envres.2009.07.012.
- Moore, J.W., & Ramamoorthy, S. (2012). Heavy metals in natural waters: Applied monitoring and impact assessment: Springer Science & Business Media., 268 pp. doi: 1461252105, 9781461252108.
- Mziray, P.K., & Kimirei, I.A. (2016). Bioaccumulation of heavy metals in marine fishes (*Siganus sutor*, *lethrinus harak*, and *Rastrelliger kanagurta*) from Dar es Salaam Tanzania. *Regional Studies in Marine Science*, (7), 72-80. doi: 10.1016/j.rsma.2016.05.014.
- Nachev, M., Schertzing, G., & Sures, B. (2013). Comparison of the metal accumulation capacity between the acanthocephalan *Pomphorhynchus laevis* and larval nematodes of the genus *Eustrongylides* sp. infecting barbel (*Barbus barbus*). *Parasites and Vectors*, 6, 1-8. doi: 10.1186/1756-3305-6-21.
- Navratil, S., Palikova, M., & Klement, R. (1999). Anguillicolosis of eels in water reservoirs of the Morava River basin. *Helminthologia*, 36, 129.
- Oyoo-Okoth, E., Admiraal, W., Osano, O., Hoitinga, L., & Kraak, M.H. (2010). Metal specific partitioning in a parasite–host assemblage of the cestode *Ligula intestinalis* and the cyprinid fish *Rastrineobola argentea*. *Science of the Total Environment*, 408(7), 1557-1562 .doi: 10.1016/j.scitotenv.2009.11.054.
- Oyoo-Okoth, E., Admiraal, W., Osano, O., Kraak, M.H., Gichuki, J., & Ogwai, C. (2012). Parasites modify sub-cellular partitioning of metals in the gut of fish. *Aquatic Toxicology*, 106, 76-84. doi: 10.1016/j.aquatox.2011.10.014.
- Pascoe, D., & Cram, P. (1977). The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, 10(5), 467-472. doi: 10.1111/j.1095-8649.1977.tb04079.
- Pascual, S., & Abollo, E. (2003). Accumulation of heavy metals in the whale worm *Anisakis simplex* s. l. (Nematoda: Anisakidae). *Journal of the Marine Biological Association of the United Kingdom*, 83(5), 905-906. doi: 224150999accountid=142908.
- Poulin, R. (1998). Evolutionary ecology of parasites: from individuals to community. Chapman and Hall Ltd., London. UK. Country of publication, London., 360 pp.
- Retief, N.R., Avenant-Oldewage, A., & Du Preez, H.H. (2009). Seasonal study on *Bothriocephalus* as indicator of metal pollution in yellow fish, South Africa. *Water SA*, 35(3), 315- 322.
- Rosenberg, D.M., & Resh, V.H. (1993). Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall Ltd., New York., 488 pp. doi: 10.1002/aqc.3270040110.
- Samuel, D., & George, P.V. (2000). Mineral constituents of freshwater fish *Channa striatus* infected with acanthocephalan parasite, *Pallisentis nagpurensis*. *Indian Journal of Fisheries*, 47(3), 215-224.
- Schulz, R.S., & Gvozdev, E.V. (1972). Fundamentals of general helminthology. Publ House Nauka, Moscow (in Russian) Snedecor, G.W, Cochran, W.G (1967) Statistical methods, 6th edn. Iowa State University Press., Ames, 251 pp.
- Shreadah, M.A., Abdel Fattah, L.M., & Fahmy, M.A. (2015). Heavy metals in some fish species and bivalves from the Mediterranean coast of Egypt. *Journal of Environmental Protection*, 6(01), 1-9. doi: 10.4236/jep.2015.61001.
- Sures, B. (2002). Competition for minerals between *Acanthocephalus lucii* and its definitive host perch (*Perca fluviatilis*). *International journal for parasitology*, 32(9), 1117-1122. [https://doi.org/10.1016/S0020-7519\(02\)00083-8](https://doi.org/10.1016/S0020-7519(02)00083-8).
- Sures, B. (2003). Accumulation of heavy metals by intestinal helminths in fish: An overview and perspective. *Parasitology*, 126(7), S53. <https://doi.org/10.1017/S003118200300372X>.
- Sures, B. (2004). Environmental parasitology: Relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology*, 20(4), 170-177. doi: 10.1016/j.pt.2004.01.014.
- Sures, B. (2007). Host-parasite interactions from an ecotoxicological perspective. *Parassitologia*, 49(3), 173-176. <http://dx.doi.org/10.1155/2012/237280>.
- Sures, B., & Roy, S. (2001). Comparison between lead accumulation of *Pomphorhynchus laevis*

- (palaeacanthocephala) in the intestine of chub (*Leuciscus cephalus*) and in the body cavity of goldfish (*Carassius auratus auratus*). *International Journal for Parasitology*, 31(7), 669-673. doi: 10.1016/S0020-7519(01)00173-4.
- Sures, B., Bahram, S.D., & Harald, F.K. (2003). The intestinal parasite *Pomphorhynchus laevis* (acanthocephala) interferes with the uptake and accumulation of lead (210 Pb) in its fish host chub (*Leuciscus cephalus*). *International Journal for Parasitology*, 33(14), 1617-1622. doi: 10.1016/S0020-7519(03)00251-0.
- Sures, B., Taraschewski, H., & Rydlo, M. (1997). Intestinal fish parasites as heavy metal bioindicators: A comparison between *Acanthocephalus lucii* (Palaeacanthocephala) and the zebra mussel, *Dreissena polymorpha*. *Bulletin of Environmental Contamination and Toxicology*, 59(1), 14-21. doi: 10.1007/s001289900437.
- Sures, B., Siddall, R., & Taraschewski, H. (1999). "Parasites as accumulation indicators of heavy metal pollution." *Parasitology Today*, 15 (1), 16-21. doi: 10.1016/S0169-4758(98)01358-1.
- Tenora, F., Baruš, V., & Prokes, M. (2002). Next remarks to the knowledge of heavy metal concentrations in gravid tapeworm species parasitizing aquatic birds. *Helminthologia*, 39(3), 143-148. doi: 10.1016/S0169-4758(98)01358-1.
- Thielen, F., Zimmermann, S., Baska, F., Taraschewski, H., & Sures, B. (2004). The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environmental Pollution*, 129(3), 421-429. doi: 10.1016/j.envpol.2003.11.011.
- Torres, M.J., Eira, C., Miquel, C.J., & Feliu, J.C. (2012). Heavy metal accumulation by intestinal helminths of vertebrates. In: Diego Muñoz-Torrero, Diego Haro and Joan Vallès (ed). Recent Advances in Pharmaceutical Sciences II, Transworld Research Network., Chapter 10, 169-181 pp.
- Uçkun, A.A., (2017). Ecotoxicological Evaluation of Pesticide Pollution in Atatürk Dam Lake (Euphrates River), Turkey, *Turkish Journal of Fisheries and Aquatic Sciences*. 17, 313-321. doi: 10.4194/1303-2712-v17_2_10.
- Velusamy, A., Satheesh, K.P., Ram, A., & Chinnadurai, S. (2014). Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. *Marine Pollution Bulletin*, 81(1), 218-24. <https://doi.org/10.1016/j.marpolbul.2014.01.049>.
- Wahbeh, M.I., & Mahasneh, D.M. (1987). Concentrations of Metals in the Tissues of Six Species of Fish from Aqaba, Jordan. *Dirasat*, 14, 119-129.
- Williams, M.A., Penlington, M.C., King, J.A., Hoole, D., & Arme, C. (1998). *Ligula intestinalis* (Cestoda) infections of roach (*Rutilus rutilus*) (Cyprinidae): immunocytochemical investigations into the salmon- and chicken-II type gonadotrophin-releasing hormone (GnRH) systems in host brains. *Acta Parasitologica*, 43, 232-5.
- Zaghloul, K.H., Abd El-Monem, S., Fayed, H.M., & Mohamed, H. A. (2001). Fish parasites as biological indicators of pollution with heavy metals. *Journal of Union of Arab Biologists*, 15, 533-561.