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Research Article

Effect of Intestinal Helminths on the Protein Content of Heavy Metals Polluted Tissues of *Lethrinus mahsena* Fish

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Abstract

Fish are valued as an ideal source of high-quality proteins, which contain large quantities of essential amino acids. The present investigation is designed to study the effect of intestinal helminths in the fish; Single, double and triple helminths infection on the protein content of fish *Lethrinus mahsena* in the presence of some heavy metals (HMs). In this study, total of 130 emperor fish were collected between November 2015 and May 2016 from the coast of Jeddah. The concentration of the HMs in the infected and uninfected fish was analyzed using the atomic flame absorption spectrometer. Total proteins were extracted from infected and uninfected fish tissues and separated by 12% of SDS-PAGE. The results revealed that individual cestodes and triple infection caused lower concentrations of As, Cd, Cu and Fe in fish tissues. While double lesions have caused a decrease in the concentration of Cd and Cu. There were no significant differences between the protein contents of infected and uninfected fish. This indicates that the presence of intestinal worms in the fortified intestine can reduce the harmful effect of accumulated HMs on the protein content of fish despite HMs contamination.

Keywords: Helminths; Heavy Metals; *Lethrinus mahsena*; Protein; Infection; Contamination

Introduction

Fish is an important source of premium animal protein with large amounts of essential amino acids. About 20,000 to 30,000 fish species have been identified and each of the species has adopted few helminthes species [1]. Environmental pollution caused by heavy metals (HMs) is a serious threat to the land and marine environment. The HMs such as cadmium (Cd) and lead (Pb) are not nutritionally necessary in high concentrations, and if found in the body at high concentrations, they are harmful to health not only for animals but also for human beings [2]. The HMs can enter fish in three ways: they can be taken directly from the surrounding waters through the skin and gill of the fish, or by absorbing toxic HMs from dirty and contaminated food. The HMs can then enter the bloodstream of fish, accumulate in the tissues of the liver of the fish, and can be biologically converted, released or consumed by humans through the food chain [3]. There are very few parasites present in the host, and it has been shown that helminths contain very high concentrations of HMs and cause toxicity [4,5]. The relationship between marine pollution and parasites can be used as a sign to check water quality, which is the most important consideration today. Intestinal parasites in fish obtain heavy mineral deposits at higher concentrations compared to the host tissues [6]. Regardless of the accumulation of natural toxins in parasites, they also play an important role in changing the fate of the chemicals present in the host [7]. Therefore, it is necessary for public health to determine the minimum concentration of HMs in the tissues of fish that humans use. The long exposure to HM pollutants interferes with the physiological, behavioral and biochemical conditions of the host and changes the state of parasites [8]. The accumulation of HMs in fish cells and tissues harms consumers, including humans. This study aimed to determine the toxicity of HMs due to single, double and triple intestinal parasitic infections on *Lethrinus mahsena* fish tissues and its effect on the protein content of infected fish tissues.

Materials and Methods

The *L. mahsena* samples were collected from Ras Mehsen, Jeddah, Saudi Arabia, contaminated with industrial waste from neighboring factories (Figure 1). From November 2015 to May 2016, 100 fish were randomly collected from the above collection site. After dissecting fresh fish, the presence of parasites was examined under a microscope. Parasites were collected and identified in the affected organs [9].

Figure 1: Map of the studied sites (google map).

Heavy metals analysis

Heavy metals were extracted from seawater using concentrated hydrochloric acid (HCl), and concentrations of Pb, Cd, Fe, Cu, As and Zn were determined according to the Ali., *et al* [10]. The samples were then analyzed using the FAAS atomic absorption test. FAAS discovered HM concentrations with Varian 5-AAS Jena Spectrometer Analysis [9,11].

Electrphoresis for protein in fish muscles

The Total protein content was extracted from the fish muscles according to Smith [12]. total protein contents were estimated according to Laemmli [13] and were resolved on 12% SDS-PAGE along with wide molecular weight standard (Sigma). Gels were imaged using the Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity 1, 4.0.3 software version.

Statistical analysis

Statistical analysis was completed by student *t*-test with SPSS (version 19).

The results

Heavy metal analysis of uninfected and infected fish

Emperor fish, *Lethrinus mahsena* muscle tissues, were examined for the existence of different types of heavy metals; As, Cd, Cu, Fe, Pb and Zn using FAAS of single Trematoda (T), Cestoda (C) and Nematoda (N), double Trematoda+ Cestoda (T+C); Trematoda+ Nematoda (T+N) and Cestoda+ Nematoda (C+N) as well as triple (T+C+N) infections, contrasted to uninfected fish.

Changes in the concentrations of HMs in fish tissues due to individual infection of the intestinal parasites are shown in table 1. Arsenic analysis showed a marked increase in its concentration in individual infected fish with T and N. The concentrations of Cd and Cu decreased significantly in affected fish. Iron decreased significantly in single fish with C and significantly increased in fish with T compared to healthy fish. Lead increased significantly in single fish tissue (C and N). The VI trace mineral analyzed was zinc. In fish tissues that infected with single C and N, the Zn concentration was significantly increased compared to healthy fish.

Uninfected	Inf. with Trematodea	*P* value	Inf. with Cestodea	*P* value	Inf. with Nematodea	*P* value
As						
386.03 ± 50.97	902.04 ± 28.20	0.000*	373.25 ± 20.45	0.129	1025.45 ± 217.37	0.001*
Cd						
0.017 ± 0.008	0.005 ± 0.002	0.000*	0.005 ± 0.004	0.03*	0.003 ± 0.001	0.008*
Cu						
0.096 ± 0.046	0.027 ± 0.013	0.057*	0.005 ± 0.001	0.020*	0.023 ± 0.010	0.026*
Fe						
2.949 ± 0.954	6.985 ± 5.029	0.018*	0.191 ± 0.071	0.019*	4.353 ± 2.558	0.248
Pb						
0.076 ± 0.009	0.103 ± 0.014	0.210	0.128 ± 0.001	0.001*	0.105 ± 0.009	0.011*
Zn						
0.948 ± 0.326	1.436 ± 0.876	0.441	3.014 ± 2.596	0.032*	3.640 ± 2.527	0.002*

Table 1: Heavy metals concentrations (mg/g) in *Lethrinus mahsena* muscle tissues single infection of Helminths.

Significant *$P \leq 0.05$.

Table 2 shows the metals concentration in the double infected fish tissues. Analysis of As in muscle tissues revealed a significantly high concentration in the double T+C and C+N infected fish. Cd in C+N infected fish tissues revealed a significantly higher concentration than healthy fish. Cadmium concentration in the muscle tissues of double (T+C and T+N) infected fish significantly decreased. Copper concentration significantly declined in double infected fish tissues. Iron was significantly lower in double (T+C and T+N) infected fish. Lead was found to be significantly increased in the fish tissues of double (T+C) infected fish. In the infected fish tissues with T+C and T+N, the Zn concentration increased significantly compared to healthy fish.

Uninfected	Inf. with (T+C)	*P* value	Inf. with (T+N)	*P* value	Inf. with (C+N)	*P* value
As						
386.03 ± 50.97	567.30 ± 195.79	0.010*	476.02 ± 140.70	0.535	821.58 ± 219.86	0.004*
Cd						
0.017 ± 0.008	0.005 ± 0.001	0.015*	0.008 ± 0.003	0.019*	0.061 ± 0.053	0.002*
Cu						
0.096 ± 0.046	0.015 ± 0.007	0.056*	0.010 ± 0.006	0.029*	0.059 ± 0.036	0.027*
Fe						
2.949 ± 0.954	0.615 ± 0.132	0.055*	0.159 ± 0.042	0.056*	2.455 ± 2.287	0.699
Pb						
0.076 ± 0.009	0.112 ± 0.010	0.011*	0.104 ± 0.015	0.109	0.099 ± 0.022	0.817
Zn						
0.948 ± 0.326	5.469 ± 2.675	0.000*	5.270 ± 2.942	0.000*	2.481 ± 1.161	0.075

Table 2: Heavy metals concentrations (mg/g) in *Lethrinus mahsena* muscle tissues in double infection of helminths

Significant *$P \leq 0.05$.

As shown in table 3, mineral analysis of triple infected fish tissues shows a significant decrease in arsenic. On the other hand, Cd, Cu and Fe were created for a big cut. Lead and zinc are significantly increased compared to healthy fish. The concentration of HMs in the collected seawater samples is: As 56.75, Zn 14.13, Pb 0.89, Fe 0.12, Cd 0.08, and Cu 0.08 mg / L.

Uninfected	Triple infection	*P* value
As		
386.03 ± 50.97	318.733 ± 47.624	0.172
Cd		
0.017 ± 0.008	0.007 ± .0.001	0.022*
Cu		
0.096 ± 0.046	0.005 ± 0.001	0.012*
Fe		
2.949 ± 0.954	0.243 ± 0.042	0.011*
Pb		
0.076 ± 0.009	0.119 ± 0.004	0.001*
Zn		
0.948 ± 0.326	4.419 ± 2.584	0.003*

Table 3: Heavy metals concentrations (mg/g) in triple infected *Lethrinus mahsena*

Significant **P*≤ 0.05.

Protein binding patterns in uninfected and infected fish

Using the total proteins extracted from uninfected and infected methylated muscle tissue, SDS-PAGE discovered some biochemical genetic differences between them. The infected fish were categorized as follows: single nematode infection (N), trematode (T) and cestode (C), double infection with T + C, C + N and T + N and triple infection (T + N + C).

As shown in table 4, in SDS-PAGE of total protein extracted from the tissues of uninfected and infected fish, a total of 20 ranges were discovered with different molecular weights ranging from 2.302 to 363.462 kDa. Seven common (single) ranges (5, 6, 9, 15, 16, 17 and 19) with molecular weights of 202.225 and 169.156, 107.222, 38.968, 26.47, 20.829 and 12.135 kDa, respectively. The resulting polymorphic bands are 14 (1, 2, 3, 4, 7, 8, 10, 11, 12, 13, 14, 18, 20 and 21), the molecular weight is 362.308, 340, 319.385, 275, 152.106, 132.697, 88.192, 77.549, 61.236, 54.843, 48.286, 16.545, 7.659 and 2.302. The results indicate that no unique group identified the infected and uninfected tissues. The tissues of the fish with trematode intestinal infection were assigned in the absence of bar numbers. This strip can be considered a negative sign for a 14-point crack infection with a molecular weight of 48,286 kDa. However, the number of protein-based phylogeny shown in figure 2 separates uninfected, single and triple infected fish in four different groups, but not completely.

NAME	M.W. KDA	U	T	C	N	T+C	T+N	C+N	T+C+N	T+C+N
BAND 1	362.308	1	0	0	1	1	0	0	1	1
BAND 2	340	1	0	1	0	1	0	0	0	0
BAND 3	319.385	0	1	1	1	0	0	0	1	1
BAND 4	275	0	1	1	1	1	0	0	1	1
BAND 5	202.225	1	1	1	1	1	1	1	1	1
BAND 6	169.156	1	1	1	1	1	1	1	1	1
BAND 7	152.106	0	1	1	1	1	0	1	0	0
BAND 8	132.697	1	1	0	0	1	1	1	0	0
BAND 9	107.222	1	1	1	1	1	1	1	1	1
BAND 10	88.192	0	1	1	1	0	0	0	1	1
BAND 11	77.549	0	1	0	0	0	1	0	1	1
BAND 12	61.236	1	1	0	0	1	1	1	1	1
BAND 13	54.843	0	1	1	1	0	0	0	1	1
BAND 14	48.286	1	0	1	1	1	1	1	1	1
BAND 15	38.968	1	1	1	1	1	1	1	1	1
BAND 16	26.47	1	1	1	1	1	1	1	1	1
BAND 17	20.829	1	1	1	1	1	1	1	1	1
BAND 18	16.545	1	1	0	0	1	1	1	1	1
BAND 19	12.135	1	1	1	1	1	1	0	1	1
BAND 20	7.659	0	0	1	1	0	1	1	1	1
BAND 21	2.302	1	1	1	1	1	1	1	1	1

Table 4: Summary of densitometric analysis for total protein profiles from uninfected (U) and infected. *Lethrinus mahsena* muscle tissues as single-infected with trematode (T), cestode (C) and nematode (N), double-infected with T+C, T+N and C+N, as well as the triple-infected (T+N+C).

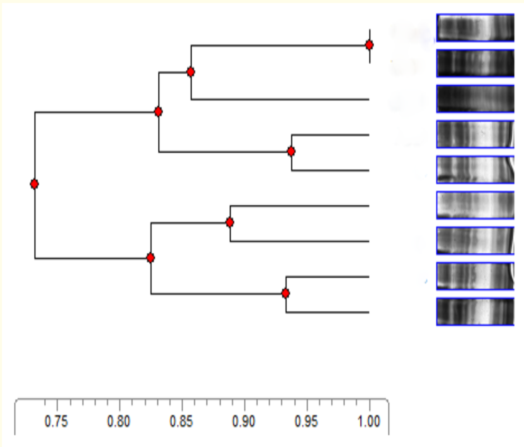

Figure 2: Protein-based neighbor-joining phylogenetic tree for uninfected (U) and infected *Lethrinus mahsena* tissues as single-infected with trematodes (T), cestodes (C) and nematodes (N), double-infected with T+C, T+N and C+N, as well as the triple-infected (T+N+C).

Discussion

Saltwater fish around the world make up the most abundant fish food. The protein content of healthy fish is considered relatively stable against clear species. In general, there are no significant seasonal differences in the protein content of fish. Parasites play a very important role in the accumulation of toxic HMs in the body. Thus, they can act as host metal basins and thus support the supply of high concentrations of HMs [14-16].

Prolonged exposure to pollutants can lead to physiological, behavioral, and biochemical changes in the host that can affect the prevalence and density of parasites [8]. Previous studies that discussed the absorption and collection of pollutants in fish tissues were achieved using fish parasites. They accumulate minerals thousands of times higher than the host's body. The internal batteries were examined for efficient metal accumulation [17].

In this study, intestinal parasites (Trematoda, Cestoda and Nematoda) were found to be single or double (Trematoda, Cestoda T + C, Trematoda, Nematoda T + N, Cestoda, Nematoda C + N) or triple. Individual infections with single, double and triple infections have a greater impact on the mineral content of fish tissues. They cause a decrease in the concentrations of As, Cd, Cu and Fe in fish infected with cestodes only, and triple (T + C + N) and decreasing in the concentrations of Cd and Cu in the double infected fish compared with healthy one.

In our previous studies, Fe, Cd, Cu and Pb concentrations were established to significantly increase intestinal parasites studied from the tissues of Emperor fish. In addition, the bioaccumulation factor of parasites was found to be significantly higher in parasites associated with fish tissues [9]. Intestinal parasites have been shown to accumulate more minerals than fisheries parasites [18]. Otherwise, cestodes can interfere with the host's metal proteins, increasing the toxicity of the metal. Poisoning HMs by absorbing not only mineral-binding proteins, but also some fats and proteins that are essential to metabolic requirements. This indicates an increase in several concentrations of HMs in the infected fish associated with the non-infected fish. The increase in HMs in fish through infection can be attributed to the competitive reaction of parasites and hosts [19,20].

Some parasites accumulate HMs in the body, which play an important role in purifying the organism. Intestinal parasites can reduce the level of HMs in Emperor fish, which can be beneficial to fish by allowing them to withstand high levels of HMs. According to previous studies, infection with nematodes appears to be good indicators of water pollution. This discovery can be strengthened by current results that confirm the parasite's ability to store HMs such as iron, cadmium, copper and lead. These data, of course, illustrate the benefits of parasites in the ocean for potential applications such as indicators for warning of HMs contamination [4,5,9].

Fluctuations in the muscle protein content due to parasitic infections include: some protein ranges disappear, the existing band disappears, or a new band presence appears. However, this difference in protein bands can be considered a reflection of the effects of stress on the levels of ribose and RNA, which influence protein synthesis [21,22].

In the present study, small differences in protein content were observed between infected and uninfected fish. These results are consistent with previous investigations and indicated that proteins in the muscles of contaminated fish will have no effect except in the case of severe loss of vitality, including glycogen and fat [23-25]. This may be due to tissue fixation techniques that serve as host methods for adapting to parasite attacks [26]. Solomon *et al.* [27], added that dual diseases have a greater impact on biochemical changes than individual pollution. The pollution resulted in three times the results of the pollution similar to the nutritional value of the fish. Triple infections were steadily strong in protein content [28].

Several studies have also addressed the relationship between HMs contamination and biosynthesis in uninfected fish. The high accumulation of HMs interferes with the biological activity of catfish by disturbing the ribosome and nucleic acids, which reduces the biosynthesis of protein, especially in muscle tissue. HMs have a limited effect on the biosynthesis of protein due to its effects on RNA and ribosome activity in uninfected catfish. High mercury and Cadmium concentrations in catfish muscles can reduce the nutritional quality of tissue proteins. Cd and Pb adversely affect increased protein efficiency in the fish body, chemical composition and muscle region in catfish [2]. Abdul-Hamid and Al-Ayouti [29] also found a negative relationship between the organic protein content and the lead content in uninfected fish. Therefore, from a public health point of view, it is important to measure the contamination of HMs from fish used in human depletion [8].

Conclusion

In the current study, we can conclude that with a single infection restricted by, all double and triple infections further affect the mineral content of fish tissues. Individual injuries and triple infection (T + C + N) caused a decrease in As, Cd, Cu and Fe concentrations in the tissues of *Lethrinus mahsena*. On the other hand, all double infection caused a lower Cd and Cu concentration compared to the uninfected. Concentrations of Cd, Cu, Fe, Pb, and Zn from uninfected *L. mahsena* were found below the SASO (Saudi Standards Organization) limits, except for As. There is a slight difference between the protein polymer strips of infected and uninfected fish, except for those with single trematodes infection. This indicates that the presence of intestinal parasites in the small intestine of *L. mahsena* can lead to the accumulation of HMs in their body, which may reduce the harmful effects of HMs on fish proteins. Parasitologists and marine environmental toxicologists must work together to conduct further research in this field.

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