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Bioaccumulation of Some Heavy Elements in Larval Stages of Digenetic Trematode

Conflict of interest: nothing to declare.
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Abstract

Introduction. The current study were conducted in one of the most important water sources in Iraq, the Tigris river.
Purpose. To study the bioaccumulation of some heavy metals (lead, nickel, zinc, copper, and cadmium) in sediments and tissues of Melanoides tuberculata shells and the redia stage of the trematode Parapleurolophocercous harbored by the snail used in the study at three stations in Misan Governorate (Al-Musharrah, Al-Salam, and Qalat Saleh).
Materials and methods. Monthly samples were collected from July until December 2022, with five replicates for each sample. The statistical results showed significant differences at the probability level of $p \leq 0.05$ between most of the heavy elements in the three study stations.
Results. The redia stages recorded cumulative concentrations of the elements higher than their concentrations in the sediments.
Conclusion. In shell tissue, all elements except lead and nickel were found to have higher concentrations in the sediment than their concentrations in the redia stage.
Keywords: heavy metals, sediments, Melanoides tuberculate, redia stage, Parapleurolophocercous, lead, nickel, zinc, copper, cadmium

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Биоаккумуляция некоторых тяжелых элементов в личинках дигенетической трематоды

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Резюме

Введение. В исследовании использовали образцы материалов, полученных из одного из важнейших источников воды в Ираке – реки Тигр.
Цель. Изучение биоаккумуляции некоторых тяжелых металлов (свинца, никеля, цинка, меди и кадмия) в отложениях и тканях улиток Melanoides tuberculata, а также в трематодах Parapleurolophocercous, для которых улитки являются промежуточным хозяином, на стадии редии. В исследовании использовали образцы, полученные на трех станциях мухафазы Майсан (Аль-Мушарра, Аль-Салам и Калат Салех).
Материалы и методы. Пробы отбирались ежемесячно с июля по декабрь 2022 года. Для повышения достоверности исследования каждая проба бралась в пяти экземплярах. Полученные результаты статистического анализа свидетельствуют о достоверных различиях (уровень значимости $p \leq 0,05$) в содержании большинства тяжелых металлов на трех исследуемых станциях.
Результаты. Выявленное на стадии редии суммарное содержание элементов оказалось более высоким, чем в отложениях.
Заключение. В тканях раковин моллюсков все элементы, кроме свинца и никеля, обнаруживаются в более высокой концентрации в отложениях, чем на стадии редии.
Ключевые слова: тяжелые металлы, отложения, Melanoides tuberculata, стадия редии, Parapleurolophocercous, свинец, никель, цинк, медь, кадмий

■ INTRODUCTION

The problem of pollution is considered one of the most important problems that have begun to take on serious environmental, economic, and social dimensions at the global level. In Iraq, the environment has been exposed in recent years to various types of pollutants as a result of wars and the increase in industrial, agricultural and commercial activities [1]. Indicated that the danger of pollutants lies in their entry into the food chain and their accumulation in the bodies of living organisms, reaching humans [2]. Hence, the need to adopt vital indicators that can be used as vital evidence of pollution, including pollution with heavy metals, becomes clear.Gastropods are considered to be the first intermediate host for trematode, as they are bioindicators and biomonitoring organisms capable of accumulating elements in their bodies at a higher level than other groups of invertebrates [3]. They are also considered to be important links in mineral cycles in the aquatic ecosystem, as some aquatic birds ingest them. And fish, which in turn reach humans through the food chain and cause health risks to them [4]. They are considered vital indicators of the health of the ecosystem and the environmental changes occurring in it [5], as most of them act as vital filters for wastewater as they feed on bacteria and other microorganisms in addition to feeding on the organic materials carried by the water [6]. Parasitic worms are sensitive to contamination with heavy metals and are characterized by their ability to bioaccumulate these elements in their bodies in various ways, especially those anthropogenic pollutants that come from humans and their various activities [7]. Therefore, they serve as an early warning, especially in low-level environmental threats that may be. They are beneficial to the health of their hosts through their ability to withdraw heavy elements from their tissues [8]. The effect of heavy metals on freshwater snails L. natalensis infection with the F. gigantica and recorded lower concentrations of Cu and Zn in infection snails compared to uninfection snails [9]. Compare concentrations of heavy metal pollution in salt marshes, before and after the snails were infection with the larval stages of trematode, it was found that the lake water recovered from pollution six years after the snails were infection with these stages [10].

The possibility of using parasites in intermediate and final hosts as vital indicators of pollution has not received sufficient attention in Iraq. As for the study of the bioaccumulation of heavy metals in the larval stages of trematode in their intermediate hosts, it is almost non-existent in the Middle East and in the countries of the world, and this is evidenced by the scarcity of studies that have dealt with.

■ PURPOSE OF THE STUDY

To study the bioaccumulation of some heavy metals (lead, nickel, zinc, copper, and cadmium) in sediments and tissues of Melanoides tuberculata shells and the redia stage of the trematode Parapleurolophocercous harbored by the snail used in the study at three stations in Misan Governorate (Al-Musharrah, Al-Salam, and Qalat Saleh).

■ MATERIALS AND METHODS

Samples collection

Collecting sediment samples. Sediment samples were collected from the river shelf that was slightly covered with water after removing the surface layer with a small shovel. A quantity of sediment was taken and placed in marked nylon bags. The sample was spread on a transparent nylon bag in the laboratory and then placed in the oven at a temperature of 75 °C until after drying it, it was ground with a ceramic mortar and sieved with a sieve with a diameter of 2 mm to remove impurities. It was stored in marked plastic containers until the digestion process was carried out according to the method approved by the Regional Organization for the Protection of the Marine Environment [11].

Collecting snail samples: picked a group of Melanoides tuberculata snails manually from each station and placed the samples in transparent plastic boxes labeled, with an amount of the same river water and some aquatic plants until they arrived at the laboratory. The snails were then cleaned of plankton such as mud, algae, and plants. The shells of the snails were broken to separate the tissue and isolate the larval stages from the infection snails. The snail tissues and the larval stages represented by the redia of the Parapleurolophocercous, which were isolated from the infection snails, were placed individually on towel paper to dry at the temperature of the laboratory, and then placed in an electric oven at 70 °C for 24 hours. Or until it dries, then it is transferred to a desiccator until it reaches room temperature, then the samples are finely ground using a ceramic mortar, and the powder is stored in clean, tightly marked plastic containers until the digestion process is carried out according to the method approved by [12] for the digestion of aquatic tissue samples.

Completely to measure the concentrations of elements under study. Measurement of heavy metal ions: The concentrations of heavy metals (cadmium, copper, zinc, nickel, and lead) were measured using a Flame Atomic Absorption Spectrophotometer, type AA7000, Shimadzu.

■ RESULTS

The statistical results indicated that there were significant differences at the probability level of $p \leq 0.05$ between most of the heavy elements in the three study stations, as follows.

Lead

The results of the statistical analysis showed that there were significant differences in the concentration of lead accumulated in sediments, shell tissue, and the Redia stage, and at the probability level $P \leq 0.05$, the highest cumulative concentration rate of lead in the sediments was recorded and amounted to 18.59 micrograms | gram dry weight, followed by the concentration rate in the rudimentary stage. Already 13.94, and conch tissue recorded the lowest value, reaching 6.52 micrograms/grams dry weight. As shown in Fig. 1.

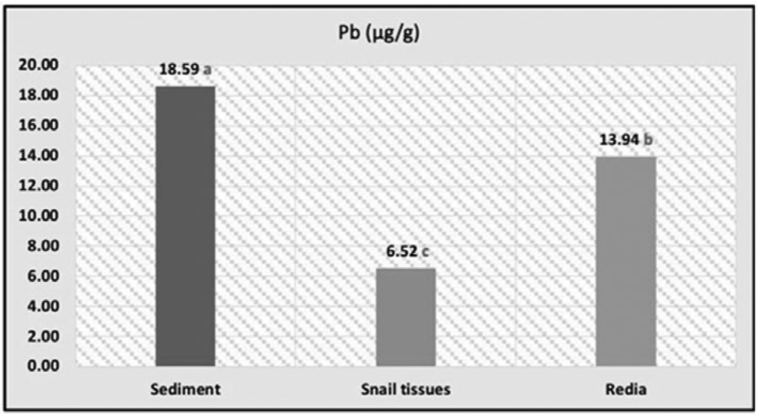

Fig. 1. Concentration of lead, estimated in micrograms/grams, in sediments, shell tissue, and redia stage

Nickel

The highest concentration of nickel in sediments was recorded at 92.57 micrograms/grams of dry weight, while the element concentration was 10.03 and 7.71 micrograms/grams in the redia stage and the shell tissue, respectively. The statistical analysis showed that there are no significant differences in the concentration rates between the redia stage and the shell tissue, while there is a significant difference between both of them and the concentration of the element in the sediment at the probability level of $P \leq 0.05$. As shown in Fig. 2.

Zinc Zn

The highest cumulative concentration of zinc in the Rhodia stage was recorded at 200.60 micrograms/grams of dry weight. The lowest concentration of the element was recorded in the sediment and amounted to 73.39 micrograms/grams dry weight. The differences were statistically significant at the probability level of $P \leq 0.05$. As shown in Fig. 3.

Copper

The highest cumulative concentration of copper was recorded in the larval stages at a rate of 41.12 micrograms/gram dry weight, followed by it in sediments at a rate of 27.81 micrograms/grams, while the lowest rate was in the shell tissue at a rate of 22.61 micrograms/grams dry weight. The statistical analysis showed that there was a significant difference between the concentration in the shell tissue and the redia stage, but the concentration in the shell tissue and sediment was not significant at the probability level $P \leq 0.05$. As shown in Fig. 4.

Cadmium

Redia stage recorded the highest cumulative rate of cadmium concentrations, reaching 1.08 micrograms/grams of dry weight, while its concentration in the shell tissue reached

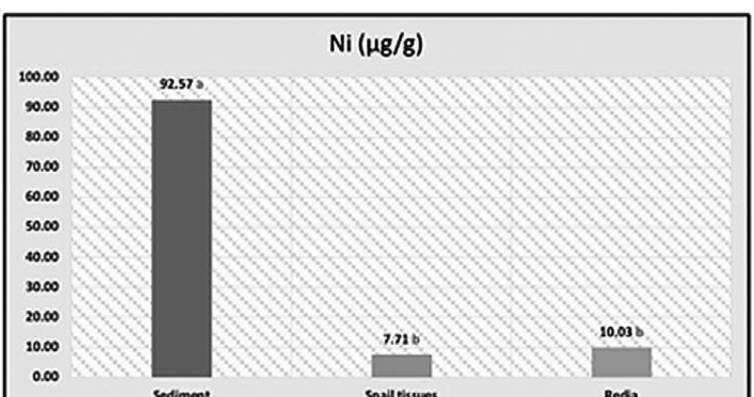

Fig. 2. Concentration of the element nickel, estimated in micrograms/grams, in sediments, shell tissue, and redia stage

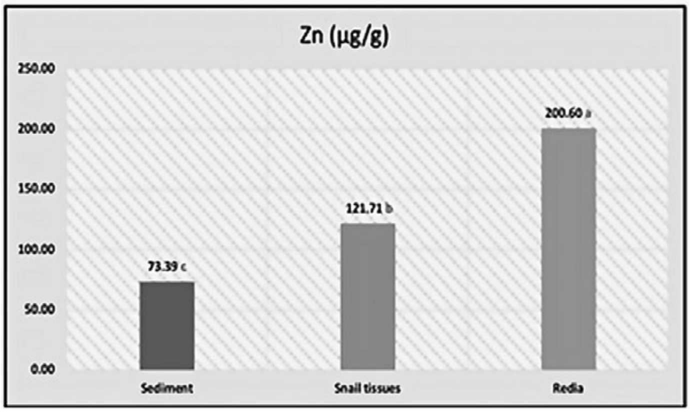

Fig. 3. Concentration of the element zinc, estimated in micrograms/grams, in sediments, shell tissue, and redia stage

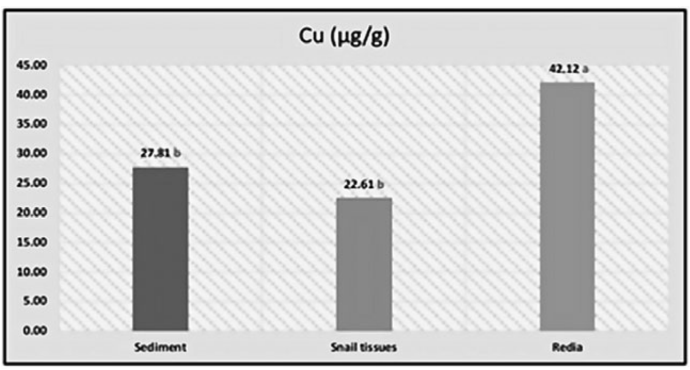

Fig. 4. Concentration of copper, estimated in micrograms/grams, in sediments, shell tissue, and rhizoid stage

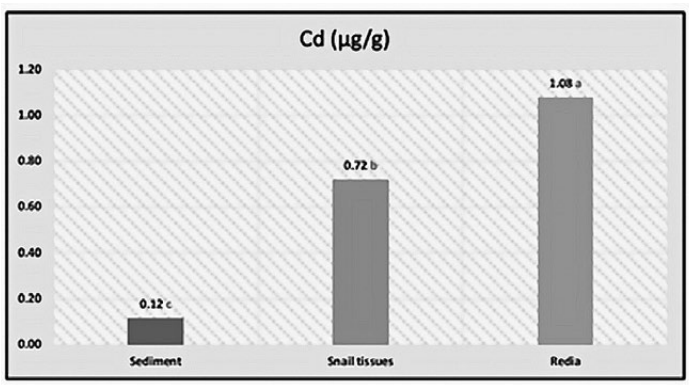

Fig. 5. Concentration of cadmium, estimated in micrograms/grams, in sediments, shell tissue, and redia stage

0.72 micrograms/grams of dry weight, and the lowest concentration rate recorded in sediments reached 0.12 micrograms/grams of dry weight. The results of the statistical analysis showed that there was a significant difference in the cumulative concentration of cadmium between the three samples under study at the probability level of P≤0.05, as shown in Fig. 5.

■ DISCUSSION

The M. tuberculata shell was chosen due to its availability of bioindicator characteristics, as this species was found throughout the study period in sufficient numbers and is easy to collect, in addition to being an intermediate host for trematode whose larval stages can be obtained most times of the year. Most studies also indicated the ability of the molluscs to concentrate many heavy elements. At relatively high levels compared to its concentration in the aquatic environment, sediments, plankton, and plants, which is why it can be used as a biological indicator. Cercaria is a small larval stage and is difficult to identify in the aquatic environment [13], so it is not possible to obtain a suitable weight from it to measure the concentrations of bioaccumulation of elements. Metacercaria is a cercaria surrounded by a primary sac in which it can grow and develop for a period. The thicker secondary sac is then secreted, and during the period of time between the secretion of the two walls, the posterior caudate can absorb heavy elements [14], but this period is very short, so it cannot accumulate heavy elements in high concentrations. Redia is the only feeding larval stage of trematode that feeds on the tissue cells of its host shell through the mouth, the muscular pharynx, and the short, unbranched intestine, in addition to feeding by diffusion through the body surface [15]. Based on the above, the redia stage was chosen in this study, which belongs to the fluke Parapleurolophocercous II, to measure the accumulation of some heavy elements Cd, Cu, Zn, Ni, and Pb, and to compare them with their concentrations in the tissues of the shell in which they originated and the environment in which the shell lives, and to demonstrate the possibility of using them as a vital indicator of contamination with these elements. .It was observed in the current study that heavy metal concentrations were recorded in the larval stages higher than in the average host in all the elements under study. This confirms that parasites are able to reduce the level of elements in the tissues of their hosts by absorbing them from the tissues of their hosts and accumulating in them [7, 16]. The results showed the highest cumulative concentration of lead and nickel in the sediment, reaching 18.59 and 92.57 micrograms, respectively. The redia stage recorded a higher concentration rate in both elements than in the shell tissue, reaching 13.94 and 10.03 micrograms, respectively, while the redia stage recorded the highest cumulative concentration of cadmium concentrations. If it reaches 1.08 micrograms grams of dry weight, while its concentration in the shell tissue reached 0.72 micrograms/grams of dry weight, and the lowest concentration rate recorded in sediments reached 0.12 micrograms/grams of dry weight. Which indicates the ability of these larval stages to bioaccumulate these elements. Lead and cadmium are non-essential elements, and their presence in high concentrations in water and their accumulation in the organisms under study indicates pollution of the aquatic environment and the impact of aquatic organisms on this pollution, which could be sourced from sewage and industrial wastewater that is thrown directly into rivers without treatment, thus causing pollution of the aquatic environment as well, on smoke emissions from vehicles and equipment running on leaded gasoline [17]. The results of zinc and copper accumulation recorded the highest concentrations in the larval stages at 200.60 and 41.12 micrograms/grams in a row. The reason for this may be attributed to emptying sewage from nearby homes and hospitals directly into rivers without treatment, especially after the spread of the Corona pandemic in previous years close to the study period, as discharging untreated sewage directly into rivers will increase the concentration of this Elements in river water, and this confirms what was indicated by [18 19]. Authors proved that comets of the family Notocotylidae isolated from the shell of L. peregra were not affected when exposed to concentrations of 0–10 ppm of Cu for a period of six weeks [20], but exposure to the same concentrations of Cu with the addition of 0.4 ppm of the element Zn led to death. Comet. This was confirmed by [12].

When they found that the comet's swimming speed and its lifespan decreased significantly when it was exposed to low concentrations of Cu and Zn together. Authors noted that comets of the Diplostomidae family had a five-hour lifespan shortened when exposed to concentrations of cadmium, compared to comets that were not exposed to the same element [22]. The elements cadmium and zinc are toxic to comets and affect their life cycle after their release from the first intermediate host, which led to a decrease in the diversity and density of metacercaria in the second intermediate hosts [21, 23]. However, another opinion when he stated that comets can swim quickly and efficiently in a polluted environment, and they can also absorb and accumulate polluted elements within their bodies [24]. The results reached by this study are consistent with the results of previous studies that dealt with the concentrations of heavy metals in parasitized worms and their ability to bioaccumulate these elements compared to their concentrations in the tissues of their hosts, including the study of [25], in which he referred to the previous result. Several times higher concentrations of lead and cadmium in tapeworms compared to the tissues of their shark hosts [26]. This result was confirmed by [27] when they found that the concentrations of lead, nickel and cadmium in parasitic worms were higher compared to their concentrations in tissues of their fish hosts indicated an increase in the concentrations of cadmium and copper in nematodes compared to their concentrations in the tissues of their fish hosts [28]. The current study confirms the value of the larval stages of trematode as sensitive bioindicators in environmental monitoring, and it is the first study of this kind. These larval stages can also be used in environmental sanitation and improving water quality from heavy metals.

■ CONCLUSION

Heavy metal pollution is common in surface water in Misan Governorate. The ability of the larval stages of trematode to accumulate heavy elements in their tissues and withdraw them from the tissues of their hosts. The possibility of using larval stages as biological evidence of heavy metal contamination. The possibility of using the larval stages of trematodes that are not important to health and veterinary health in sanitating the aquatic environment and improving its quality by drawing environmental pollutants into the tissues of the larval stages.

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