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Bioaccumulation of heavy metals in *Bothriocephalus acheilognathi* cestoda and the definitive host, *Cyprinus carpio* (L. 1758)

B.H. Al-Niaeemi[1] and M.H. Dawood[2]

[1]Department of Biology, College of Sciences, [2]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Mosul, Mosul, Iraq

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Correspondence:
M.H. Dawood
maruadaood@uomosul.edu.iq

Abstract

Concentrations of three heavy metals as Manganese, Nickel, and Cobalt were estimated in two levels in the food chain; the omnivorous *Cyprinus carpio* fish and its intestinal cestode *Bothriocephalus acheilognathi* as end consumer (endoparasite) using atomic absorption technique. The study was performed in two locations in Tigris River, Al Rashedia and Sherikhan villages/Mosel City/ Nineveh Province between June 2022 to October 2022. The concentration of the three chosen metals: Mn, Ni, and Co, was estimated in the liver, gills, intestine, and skeletal muscles in both infected and uninfected fish and added to tissues of the cached Cestoda. Manganese concentration was the highest in the gills of both infected and uninfected fish 14.597, 21.773 µg/gm fresh weight, nickel concentration was the highest in the liver 4.44 and 8.10µg/gm fresh weight, and cobalt concentration was the highest in the intestine 2.467 and 7.79 µg/gm fresh weight. The difference in values was significant at $P \leq 0.05$ in the infected and uninfected fish, respectively. Accumulation of the three metals Mn, Ni, and Co was the lowest in fish skeletal muscles. Mn had the highest accumulation mean in fish organs 11.846 µg/gm fresh weight, Ni was the next 4.094 µg/gm, and Co was the lowest 2.616 µg/gm. The concentration of Mn and Ni in the cestode *B. acheilognathi* tissues 22.53 and 10.45 µg/gm was about two folds of that found in its host fish *C. carpio*. The concentration of Co was approximate in the worm tissues and its host fish. In conclusion heavy metals in *C. carpio* didn’t exceed the WHO and the FAO set permissible levels. *B. acheilognathi* cestoda could be a useful bioindicator for heavy metal contamination in aquatic ecosystems.

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Introduction

Heavy metals are considered a major class of water pollutants that may disturb the equilibrium in aqueous environments, impacting the variation of living organisms, including fish (1). Commonly, organisms require some heavy metals in trace amounts, even as co-factors in biochemical pathways or as essential molecules in cell construction. Unfortunately, non-illegible accumulation of heavy metals in the aqueous environment may result from human activities (2). The extent of accumulation in living bodies may depend on the metal's concentration in the surrounding environment, exposure time, temperature, salinity, feeding habits, and physiological state according to sex, age, and health status (2). The increasing elevation in heavy metals may lead to dire results since these metals have long half-lives, are difficult to disintegrate, could be spread away from the source of their emanation, and can accumulate in the tissues of living organisms at a toxic level (3). Monitoring the levels of these metals, especially in aquatic environments, is necessary. Fish are distinguished members of the aqueous communities in their tolerance to heavy metals; they either get them directly from the ingested food or indirectly through their gills. As the next predator eats the fish, the concentration of heavy metals would biomagnify through the food chain (4). Thus, fish has been used as bioindicators to estimate water pollution with heavy metals and other organic compounds (5). The muscle of *C. carpio* fish tissue was not safe for human consumption and that the groundwater in the Khor al-Zubair area in Basra governorate/South Iraq is possibly contaminated with the heavy metals: Cr, Ni, Hg, Pb, and Cd, mainly owing to industrial activity (6). Manganese (Mn): Mn is found naturally in water and soil, almost accompanied by ferrous. Mn is an essential element living organisms use as an enzyme activator and aids in bone hardening in vertebrate animals. Elevated amounts of Mn in the environment may result from the manufacture of batteries, plant fertilizers, and the preparation of alloys (3). The Nickeil (Ni): Nickeil does not exist freely in nature and is combined with other metals. It is needed in trace amounts to activate some enzymes in living organisms, added to its role in ferrous absorption and, thus, hemoglobin formation. The abnormality of Ni's existence in an aqueous environment may relate to fuel and industrial wastes (7). The Cobalt (Co): In living organisms, trace amounts of Co are necessary for metabolic activity, like carbohydrate and protein metabolism, and work as activating factor in several enzymatic and immunological cellular reactions, in addition to being included in the composition of vitamins B_{12} (8). Some workers set their sights on the accumulation of heavy metals in fish and their parasites, the impact of these metals on the parasite distribution, and host physiology (9-13).

The present work was aimed to investigate the bioaccumulation of three heavy metals (Mn, Ni, Co), those expected to be found in Tigris water in concentrations over the permissible limit, since they are exchanged into the river, especially with dairy and soft drinks factories. Organisms of two levels in the food chain were Chosen, *Cyprinus carpio* fish, and its intestinal Cestoda, *Bothriocephalus acheilognathi*. The two living organisms are setter populations in the water of Mosul`s Tigris (Within Nineveh Governorate).

Materials and methods

Ethical approve

University of Mosul, College of Veterinary Medicine, the Institutional Animal Care and Use Committee, give the final acceptance to conduct experiment on animals numbered UM.VET.2023.015, sated 18 march 2023.

Study location

The study was performed in two locations in the Tigris River, In Al Rashedia and Sherikhan villages (about 6 km Northwest of Mosel City/ Nineveh Province) between June 2022 to October 2022. Both sites are densely populated, adding farms, poultry, and livestock fields to hospitals and healthcare centers. The two locations also contain power stations, dairy, and soft drink factories. As well as being residential areas. It seems that wastewater disposal is irregular there and discards directly to the Tigris River.

Sample collection

A 52 *C. carpio* fish were hunted using fishnet, and their weight ranged from 1-1.5 Kg. The fish then desiccated according to Dybem (13), locking for the intestinal cestode *Bothriocephalus acheilognathi* in the small intestine. Furthermore, four body organs were isolated from each hunted fish after killing (Gill, liver, intestinal muscles, skeletal muscles) either the fish was infected or uninfected with the intestinal cestodes.

Sample preparation

The isolated tissues and the cached cestodes were washed with Distilled water and put on filter paper to eliminate excess water. Each sample's Fresh weight was recorded, then frozen at -20ºC in a locked container until the next steps of the research work.

Estimation of heavy metals concentration

The concentration of the three chosen metals: Mn, Ni, and Co, was estimated in Fish and helminth tissues. The targeted cestoda *Bothriocephalus acheilognathi* was collected from fish after making longitudinal fissures along the fish intestine, put in a Petri dish, and washed three times with PBS at pH 7.3. After insurance of the Cestoda identity under a dissecting microscope, the fresh weight of each collected helminth was recorded. The isolated fish tissues and the helminths were digested according to (14). A 1 ml of concentrated HNO_3 65% was added to 0.1 gm of each sample. Homogenizer was used to disintegrate the samples, and then the sample was put in a glass test tube with a tide lid and incubated at 70°C in the water bath for 24hrs; the tubes were then left for 72 hrs. to complete tissue digestion. To determine the heavy metal concentration in each sample, a colorimetric method was performed using Atomic Absorption Spectrophotometer (Perkin Elmer-4000 USA.). The concentration of each metal under study (µg of the metal/gm. fresh weight) was estimated depending on a standard curve of the metal (14).

Statistical analysis

Complete randomized design, Duncan multiple range tests, and T-tests were used to compare compatibility and differences between values mean (15). All differences were considered significant at $P \leq 0.05$.

Results

Bioaccumulation of some heavy metals commonly discharged to the Tigris River in Mosul City was estimated in the present work. *C. carpio* fish, and its intestinal cestoda, *B. acheilognathi*, were chosen as they are two setter populations in an ecosystem.

Table 1 illustrates the mean concentration of Mn in uninfected and infected *C. carpio* fish. The highest concentration of Mn was observed in gills 21.773 and 14.597 µg/gm fresh weight, followed by the liver 16.157 and 12.123 µg/gm fresh weight, intestine 12.607 and 9.11 µg/gm fresh weight, and skeletal muscles 5.597 and 2.8 µg/gm fresh weight, both in uninfected and infected fish; respectively. The different between values were significant at P≤0.05.

Table 1: Bioaccumulation of Manganese in fresh weight of *Cyprinus carpio* fish internal organs

Body Organ	Mean ± SD (µg/gm) in fresh weight of *C. carpio* organs (n=3)		
	uninfected fish	Infected fish	Mean
Gills	21.773^{A}±0.989	14.597^{B}±0.032	18.185
Liver	16.157^{B}± 0.050	12.123^{C}±0.015	14.140
Intestine	12.607^{C}±0.025	9.11^{D}±0.1	10.858
Muscles	5.597^{E}±0.070	2.8^{F}±0.01	4.199
Mean	14.034	9.6575	11.846

According to the Duncan test, Different letters refer to significant differences between values at P≤ 0.05.

In table 2 mean concentration of Ni both in uninfected and infected *C. carpio* fish was listed. The highest concentration of Ni was observed in the liver 8.10 and 4.44 µg/gm fresh weight, followed by the gills 5.393 and 3.41 µg/gm fresh weight, intestine 4.517 and 2.793 µg/gm fresh weight, and skeletal muscles 1.90 and 2.20 µg/gm fresh weight, both in uninfected and infected fish; respectively.

Table 2: Bioaccumulation of Nickel in fresh weight of *Cyprinus carpio* fish internal organs

Body Organ	Mean ± SD (µg/gm) in fresh weight of *C. carpio* organs (n=3)		
	uninfected fish	Infected fish	Mean
Gills	5.393^{B} ±0.349	3.41^{D} ±0.02	4.402
Liver	8.10^{A} ±0.046	4.44^{C} ±0.046	6.270
Intestine	4.517^{C}± 0.130	2.793^{DE}± 0.025	3.655
Muscles	1.90^{E} ±0.01	2.20^{E} ± 0.026	2.050
Mean	4.839	3.211	4.094

According to the Duncan test, Different letters refer to significant differences between values at P≤ 0.05.

Table 3 illustrates that Co was highly accumulated in the intestine 7.467 and 4.790 µg/gm fresh weight of *Carpio* fish, and to a less extent in the gills 4.507 and 1.663 µg/gm fresh weight, then liver 2.133 and 1.823 µg/gm fresh weight and skeletal muscles 1.007 and 0.217 µg/gm fresh weight, both in uninfected and infected fish; respectively.

Table 3: Bioaccumulation of cobalt in fresh weight of *Cyprinus carpio* fish internal organs

Body Organ	Mean ± SD (µg/gm) in fresh weight of *C. carpio* organs (n=3)		
	uninfected fish	Infected fish	Mean
Gills	4.507^{B} ±0.144	1.663^{D}±0.120	3.085
Liver	2.133^{C}± 0.142	1.823^{D}±0.025	1.978
Intestine	7.467^{A}±0.104	4.790^{C}±0.083	6.129
Muscles	1.007^{D}±0.095	0.217^{E}±0.031	0.612
Mean	2.529	2.703	2.616

According to the Duncan test, Different letters refer to significant differences between values at P≤ 0.05.

Table 4 displays the concentration of the metals; Mn and Ni, and Co in *Bothriocephalus acheilognathi* tissues 22.53, 10.45, and 2.067 µg/gm fresh weight, respectively. It is observed that the concentration of Mn and Ni was higher in helminthic tissues than in tissues of the host fish (Figure 1). It is worth knowing that the concentration of both Mn and Co was higher in fish muscles of uninfected fish 5.597 and 1.007 µg/gm fresh weight, respectively than in muscles of infected fish 2.80 and 0.217 µg/gm fresh weight, respectively. The concentration ion of Ni was higher the in the muscles of infected fish 2.20 µg/gm fresh weight tin than in the uninfected fish 1.90 µg/gm fresh weight.

Table 4: Bioaccumulation of manganese, nickel, and cobalt µg/gm fresh weight of *Bothriocephalus acheilognathi* tissues

Heavy metal	Mean ± SD
Mn	22.53^{*}± 0.369
Ni	10.45^{*}± 0.292
Co	2.067^{*}±0.057

* Refer to the presence of significant differences between treatments at P≤0.05, according to T-test.

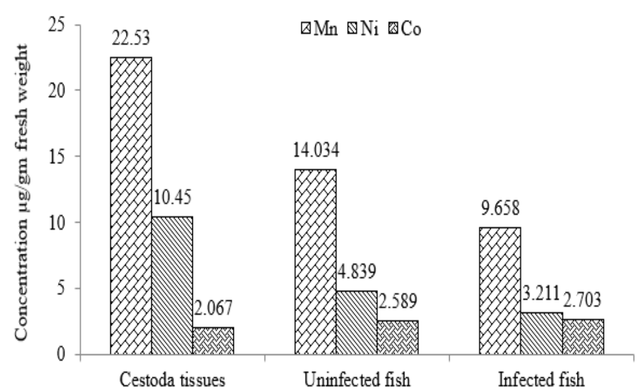

Figure 1: Variation in concentrations (µg/gm fresh weight) of the three heavy metals (Mn, Ni, and Co) in *B. acheilognathi* cestoda and uninfected and infected *C. carpio* fish, respectively.

Discussion

The present study aimed to evaluate the biomagnification possibility of several heavy metals commonly discharged into the Tigris River dairy. Two locations in Mosul distinct were chosen, Al Rashedia and Sherikhan villages. These villages are known to be important regions for industrial, agricultural, and other relhumanan activities. The study was conducted on two living organisms interconnected by a parasitic relationship. *B. acheilognathi* is a cestode recorded as an intestinal parasite for the common carp *C. carpio* living there (16). The parasitic cestode has a distinct location in the food chain as an internal consumer. It could be a unique model for estimating the bioaccumulation of a particular pollutant like heavy metals.

The concentration of the three chosen metals was first estimated in four metabolically active tissues in the fish, then estimated in tissues of the intestinal cestode as illustrated in up worded results.

The results agreed with that of Al-Weher (17) those that recorded the highest concentration of some metals in the gills of *C. carpio* uninfected fish and the lowest concentration in skeletal muscles. Elwasify *et al.* (18) also recorded a higher concentration of Mn in the Gills of *Tilapia zillii* fish (Settled in Qarun Lake in Egypt) and the lowest in skeletal muscles. On the other hand, recent results do not agree with that of Tekin-Özan and Barlas (19), which showed that the highest accumulation of Mn in *Tinca tinca* fish (Settled in Beysehir Lake in Turkey) was observed in the liver, then gills and skeletal muscles.

Gills are directly exposed to pollutants found in water because of their structure and physiology; thus, the highest concentration of heavy metals offset with the highest accumulation of that metal in fish gills (20). Karadede *et al.* (21) were referred to that the heavy metals that can combine with mucous materials in gills forming hardly removable complexes. Yousafzai *et al.* (22) combined the attraction between the positively charged heavy metals and the negatively charged phospholipids found in the mucous lining epithelium of fish gills.

On the other hand, Hantoush *et al.* (23), which illustrated that Ni was highly concentrated in the liver of silver carp fish (collected from the farms of Middle Iraq). Bhuvaneshwari *et al.* (24) was referred to the high accumulation of Ni in the liver of *Oreochromis mass amicus* fish 25.67 µg/gm fresh weight, then in gills 12.5 µg/gm and muscles 4.5 µg/gm in Kaveri River in India. Arantes *et al.* (25) estimated the accumulation of heavy metals in *Pseudoplatystoma correct* fish in (Paraopebia River in Brazil), and so Rajeshkumar and Xiaoyu (26) those referred to the high accumulation of heavy metals in the fish liver in Taihu Lake in China.

The higher concentration of Ni in liver tissues is reliable because the liver is a metabolically active organ in which several biochemical pathways are performed (27). It is the first organ that receives metabolites from the intestine through blood and lymphatic circulation. Heavy metals also arrive in liver tissue in bloodstream, then either removed with bile salts, discharged to the small intestine and then to the outer environment, or bound with some side groups available in the liver like carboxyl, amino, sulfate, nitro, and mercapto groups, added to metallothionein (28). Some of these metalorganic complexes are reabsorbed in the small intestine and stored in the liver (29).

Yousafzai *et al.* (22), which concluded that the concentrations of heavy metals were higher in the intestine of *C. carpio* fish than in other body organs. They excluded that Co metal inter fish body during nutrition rather than respiration (through gills). Elsenhans *et al.* (30) referred to the tide combination of ingested metals with the mucous membrane in the fish intestine that make it hard to remove these metals. Furthermore, some heavy metals attached to mucous membranes could be absorbed by intestinal villi after a while; other heavy metals could be discharged with fecal materials (30).

The present work revealed that the body organs of fish differ in their deterioration to heavy metals. Mn, Ni, and Co were found in the lowest concentration in muscles. This result agreed with that of Akinsanya and Kuton (12), Tekin-Özan and Barlas (31), Yilmaz and Aldhamin *et al.* (32), who concluded that heavy metals accumulated in skeletal muscles to at less extent than in other fish organs. This status result is fortunate since the skeletal muscles of fish are put on the main food lists for humans and the mean concentrations of the studied metals, Mn, Ni, and Co, did not reach the lower permitted levels of such metals in fish tissues by FAO and FDA 4.4-7.9µg/gm of fish muscles for Mn, 17.8-20 µg/gm for Ni and 2.6-5.39µg/gm for Co (33). Besides, we must consider that the over threshold accumulation of heavy metals in fish muscles may hazard human health (22,34). The relatively low concentration of heavy metals in fish muscles may indicate the weak or disability of muscle proteins to combine with these heavy metals (35). Furthermore, Perimusculer connective tissue around skeletal muscle bundles contains small amounts of lipids, the favorable binding site for heavy metals (36).

It is worth knowing that the concentration of both Mn and Co was higher in fish muscles of uninfected fish 5.597 and 1.007 µg/gm fresh weight, respectively than in muscles of infected fish 2.80 and 0.217 µg/gm fresh weight; respectively. The concentration of Ni was higher in the muscles of infected fish 2.20 µg/gm than in the uninfected fish 1.90 µg/gm. The total concentration mean of Mn 11.846 µg/gm was higher than that of Ni 4.094 µg/gm and Co 2.616 µg/gm, respectively. The variation in concentration of metals may relate to the fact that body tissues and organs differ in their reactions toward each metal, either discharging or accumulating, or interacting with the heavy metal (34). Duration and extent of exposure to the metal added to the portal of entry to fish tissues, climate changes (like pH, salinity, and temperature) in the aqueous environment also affect factors (37-39).

The result came to agree with that of Hassan, *et al*. (1), Kirin and Kuzmanova (11), Akinsanya and Kuton (12), Eira *et al.* (40), and Oyoo-Okoth *et al.* (41), those who concluded that the concentration of heavy metals in parasitic helminths is almost higher than in tissues of their host fish. As for Co, its concentration was approximately the same in the host fish and the parasitic helminths. The higher concentration of heavy metals in parasitic helminths compared to its host fish may be referred to the biomagnification of these metals through the food chain, concerning that the parasite is consumed by the fish internally. This is evident especially in metals that enter the body with engulfed water and food since the worm is an intestinal parasite. This interpretation agrees with the opinion of Chowdhury *et al.* (42).

Cestodes that inhabit fish intestines have no digestive system and thus absorb ready nutrients from fish intestinal contents, including the swallowed heavy metals, throughout the helminth tegument. The concentration of heavy metals would be higher in helminth tissues than the fish tissues, especially if the fish ingest nutrients rich with fatty materials (43). Furthermore, intestinal cestodes were found to be able to accumulate different heavy metals in their tissues at a variable level. This may relate to host species, type of host food, age, and absorptive surface area of the parasitic cestoda (44).

Conclusion

Mn hit the highest accumulation both in the body of *C. carpioo* fish and *B. acheilognathi* cestoda, followed by Ni and Co. There was selectivity in the accumulation of the three metals in different body organs of common carp. The highest concentration of Mn was found in gills, Ni was more concentrated in the liver, and Co was relatively more accumulated in the small intestine. Less accumulation of the three metals was observed in skeletal muscles. The concentration of the three metals was less in the tissues of the infected fish than in those uninfected. The concentration of both Mn and Ni was duplicated in cestoda tissues compared with the host fish tissues, but not Co, which had an approximate concentration in fish and cestoda tissues.

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Conflict of interest

None included.

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التراكم الحيوي للمعادن الثقيلة في الدودة الشريطية بوثريوسيفالس اكيلوكناثي ومضيفها النهائي اسماك الكارب الاعتيادي

بشرى حسن النعيمي[١] و مروة هاشم داؤد[٢]

[١]فرع علوم الحياة، كلية العلوم، [٢]فرع العلوم السريرية المختبرية، كلية الصيدلة، جامعة الموصل، الموصل، العراق

الخلاصة

قدرت تراكيز المعادن الثقيلة الثلاثة، المنغنيز والنيكل والكوبلت، في مستويين ضمن السلسلة الغذائية: اسماك الكارب الاعتيادي سايبسرس كاربيو والدودة الشريطية بوثريوسيفلس اكيلوكناثي باستخدام تقنية الامتصاص الذري. اجريت الدراسة في مياه نهر دجلة المار بقريتي الرشيدية وشريخان في قضاء الموصل/ محافظة نينوى خلال الفترة بين حزيران٢٠٢٢ الى تشرين الاول ٢٠٢٢. قدر تركيز المعادن الثلاثة: المنغنيز والنيكل والكوبلت في اعضاء الكبد والغلاصم والامعاء والعضلات الهيكلية لكل من الاسماك المصابة وغير المصابة فضلا عن انسجة الدودة الشريطية. بينت الدراسة بان اعلى تركيز معنوي للمنغنيز كان في الغلاصم١٤,٥٩٧ و ٢١,٧٧٣ ميكروغرام/ كغم وزن طري، النيكل في الكبد٤,٤٤ و ٨,١٠ ميكروغرام/ كغم والكوبالت في الأمعاء ٢,٤٦٧ و ٧,٧٩ ميكروغرام/ كغم لكل من الأسماك المصابة وغير المصابة على التوالي. في حين كان اقل التراكيز للمعادن الثلاثة في العضلات الهيكلية للأسماك. عموما فان اعلى تراكم للمعادن الثلاثة في

انسجه الأسماك كان للمنغنيز ١١,٨٤٦ ميكروغرام/ كغم، تلاه النيكل ٤,٠٩٤ ميكروغرام/ كغم وأخيرا الكوبلت ٢,٦١٦ ميكروغرام/ كغم. لوحظ من الدراسة أيضا أن تركيز المنغنيز والنيكل في أنسجة الدودة الشريطية ٢٢,٥٣ و ١٠,٤٥ ميكروغرام/ كغم، كان ضعف التراكيز المسجلة في أعضاء المضائف النهائية للأسماك، أما تركيز الكوبلت في أنسجة الدودة فكان مقاربا لما سجل في أعضاء مضيفها. نستنتج من هذه الدراسة بان التراكيز المسجلة للمعادن الثلاثة في اعضاء الاسماك كانت ضمن الحدود المسموحة بها من قبل منظمتي الصحة العالمية والأغذية العالمية، وان الدودة الشريطية بوثريوسيفالس اكيلوكناثي تعد كمؤشر حيوي جيد لتقييم مدى التلوث بالمعادن الثقيلة في البيئة المائية.