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ESTIMATION OF SOME HEAVY METALS BIOACCUMULATION IN TISSUES OF THE INTESTINAL CESTODA *POSTGANGESIA ARMATA* AND ITS DEFINITIVE HOST; THE EUROPEAN CATFISH *SILURUS GLANIS* (L.) FROM TIGRIS RIVER PASSING THROUGH NORTHERN N. GOVERNORATE, IRAQ

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ABSTRACT: Objective: The purpose of the study is to pursue some heavy metals in the aqueous environment of Tigris river that passes through Mosul city prior to the bioaccumulation of such metals in catfish *Silurus glanis* and its intestinal tapeworm *Postgangesia armata*. **Methods:** *Silurus glanis*, a freshwater fish were hunted from Tigris river passing through Al-Rashidiya region/Nineveh Governorate, North Iraq, during November 2017 and May 2018. Bioaccumulation of Fe, Cr, and Mn were estimated in different body tissues of infected and uninfected catfish, and in catfish intestinal tapeworm *P. armata*. Atomic absorption Spectrophotometer was used to estimate heavy metal concentration as µg of a particular metal/gm of fresh weight. **Results:** Concentration of the metals in catfish tissues were in the order of Mn > Fe > Cr. Whereas, concentration of the metals of *the tapeworm P. armata* were in the order: Fe > Mn > Cr. Moreover, mean concentrations of the three heavy metals (Cr; Fe and Mn) were lesser in body tissues of infected catfish than in the uninfected fish. This lessening of metals accumulation in the infected fish may be related to the presence of the intestinal tapeworm. It also appeared that the three metals are mostly accumulated in liver of the catfish, then in gills, and muscles at lesser extent. **Conclusions:** Present work is drawn out that measuring concentration of heavy metals in parasitic and host tissues that inhabit a certain aqueous environment could be adopted as bioindicators for assessing heavy metals pollution.

INTRODUCTION: Water is the fundamental composite of life and the most important requisite for civilizations emergence and persistence. As for the Tigris river, it is the most important water source in Mosul city/North Iraq.

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It is adopted for many humane activities. Additionally, it has momentous role in the maintenance of the aquatic community in such region of the city. Tigris river gets in Mosul city at the North-westward side, bisecting the city to Right and Left coasts. Different industrial and agricultural residuals, as well as residential and hospital heavy water, all are directly or indirectly discarded in such area of Tigris river. Al-Rawi (2005) [1] reported that the average of discarded materials in Tigris river that pass through Mosul city is 350.000M^3/day.

Physical and Chemical properties of the aquatic culture have the main role in typing, distributing and acclimating of living organism in the culture [2]. Any aquatic culture could be exposed to different pollutants, either biological or chemical that may change the environmental equilibrium in such culture, and thus affect the whole lifestyle in such regions [3].

Heavy metals are one of the important pollutants of aquatic cultures, those may be liberated to the environment naturally through geochemical cycling or may discharge to the water from different sources, the anthropogenic activities may lead to environmental misbalance [4]. Each heavy metal has lower and maximum limits in the aquatic cultures, that should be periodically checked up, since, incremental concentrations of heavy metals through different food chains in such cultures often have passive effects on the living model of the aquatic communities. On the other hands, heavy metals have the ability to bioaccumulate and concentrate through the food chains [5, 6, 7]. Al-Sanjary *et al*., (2008) [8] referred to the high value of different heavy metals in the mouth of Al-Khoser river that poses toward Tigris river In Mosul city. Abdul-Jabbar (2008) [9] stated that residual heavy metals in Tigris water in Al-Naseria city-North Iraq, inhibit bio-hydrogenation and bio-oxidation processes during water remediation.

Heavy metals were put at the head of factors which should be monitored. Since, they may lead to environmental contamination of marine communities and could disrupt the natural balance between organisms in ecosystems [10]. Biomonitoring heavy metals contamination in the aquatic cultures could be aided depending on different means. And since that fish, and thus their endoparasites, have low capability for elimination and detoxification of such metals and other chemical contaminants, and finally undergo bioaccumulation and bio-concentration of such materials [11, 12]. Thus estimation of heavy metals in fish tissues was adopted by several researchers as a marker for evaluating water pollution in Tigris river that passes through Mosul city [5, 6, 13].

Chromium (Cr) is a grey, hard metal, most commonly found in the trivalent state (III) in nature. Hexavalent (chromium VI) compounds are also found in small quantities [14]. Chromium (III) is recognized as a trace element that is essential to both humans and animals. Chromium (VI) compounds are toxic, carcinogenic have mutagenic and systemic effects in humans [15]. Chromates with low water solubility are mainly cleared to the gastrointestinal tract, whereas more soluble chromates are absorbed into the blood [16]. Chromium (III) has low solubility in water and thus absorbed to a greater extent than chromium VI on the respiratory surface [17]. Toxic effects of chromium VI in fish include hematological, histological and morphological alterations, inhibition/reduction of growth, production of reactive oxygen species (ROS) and impaired immune function [18].

Manganese (Mn) is an essential element for humans, animals, and plants and is required for growth, development, and maintenance of health. It is comprising approximately 0.1% of the earth's crust, and the twelfth most abundant element and the fifth most abundant metal involved in many chemical processes in the body, including the processing of cholesterol, carbohydrates, and protein. It is also might be involved in bone formation. Mn could be released into the air from mining and manufacturing operations and combustion of gasoline additives [19].

Iron (Fe) is a metallic chemical element that is found widely in our planet. It is mainly supplemented through diet, further classified as heme and non-heme iron. It has an important role in hemoglobin formation, muscle and brain function, and regulation of body temperature. It participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport [20].

Aim of the Study: to estimate concentrations of some heavy metals including Cr, Fe, and Mn in different body tissues of the freshwater catfish, *Silurus glanis*, either those infected or uninfected with the intestinal tapeworm, *Postgangesia armata*. Evaluating the bioaccumulation of these heavy metals in tapeworm tissues comparing with their definitive host, the catfish. Then shows the ability to use such application as bioindicator for water pollution with such metals.

MATERIALS AND METHODS:

Description of Studying Zone: The study is applied in Al-Rashidiya region which is distance 4Kms Northwestward Mosul city center/Nineveh province/Iraq Republic. In such town, high voltage power plant and water treatment plant were constructed. In addition to the presence of dairy factory, gas, and spirituous beverage factories. Moreover, Al-Rashidiya is an agricultural area that holds poultry farms, plant livestock fields, and farmlands. Besides, it is a populated area in which relatively large rivulet for water disposal is passing and directly outpouring in Tigris river stream. Herewith, it was necessary to evaluate water pollution in such area.

Sample Collection: European catfish *Silurus glanis* were hunted from Tigris river that passes through Al-Rashidiya region, in the period between November 2017 and May 2018. They hunted fish then brought to the lab., dissected according to Dybem method (1983) [21]. Gills, liver, and muscles were isolated from each fish. Furthermore, intestine of each fish was isolated and dissected looking for the intestinal tapeworm *Postagangesia armata* [22]. The helminthes chosen for the subsequent study were of the same length and weight, disregarding their age. The isolated catfish tissues and the tapeworm were washed several times with distilled water, dried with filter paper, then transferred to clean, dry, tightly closed cases, preserved in deep freeze (-20 ºC) for the time being used.

Sample Preparation for Heavy Metals Analysis: 0.2 gm from each sample which isolated previously (Gills, liver, and muscles of infected and uninfected catfish, in addition to tapeworm tissues) were put in graduated container (15 ml capacity). 1 ml of digestive fluid was added to each sample (digestive fluid: concentrated Nitric, sulfuric and perchloric acids (1:1:1). Each container then tightly closed, left for 12-72 h at room temperature to complete tissue digestion. Then de-ionized distilled water was added to each sample to complete the volume to 10 ml. Each digested sample was filtered using membranous filters. The filtrates then kept for subsequent analysis [6].

Heavy Metals Estimation: Concentrations of Fe, Mn and Cr were estimated in each previously prepared sample using Atomic Absorption Spectrophotometer (Perkin Elmer-4000 USA). Absorbance values were converted then to concentrations (µg of the particular heavy metal/gm of the sample fresh weight). Estimation for each metal Concentration was depending on the standard curve of the metal under study [23].

Statistical Analysis: Complete randomized design and Dunkan's multiple range test were applied to compare the differences and compatibility between treatments mean [24]. All results were considered significant with P≤0.05.

RESULTS AND DISCUSSION: In the present work, three heavy metals (Cr, Fe, and Mn) were tested for their bioaccumulation in body organs of catfish and in tissues of the intestinal tapeworm *Postgangesia armata* that parasitized the catfish as bioindicators for water pollution in Tigris river-Mosul city. Statistical analysis of data using Annova table and Duncan`s multiple tests revealed a significant interaction between different values at P≤0.05 that belonged to infected and uninfected catfish. **Table 1** showed that the mean concentration of Cr in uninfected catfish (6.224 µg/gm fresh weight of catfish organs) was higher than that found in infected catfish (5.041 µg/gm). On the other hand, mean concentration of Cr in liver (8.606 µg/gm) was higher than that in gills (8.083 µg/gm) and that found in catfish muscles (0.209 µg/gm).

TABLE 1: MEAN CONCENTRATION OF CHROME (Cr) µg/gm FRESH WEIGHT OF CATFISH BODY ORGANS

Body Organ	Mean conc. of Cr µg/gm fresh weight of catfish organ		
	Uninfected fish	Infected fish	Mean
Liver	8.103^{C}	9.110^{B}	8.606^{A}
Gills	10.210^{A}	5.956^{D}	8.083^{B}
Muscles	0.360^{E}	0.058^{E}	0.209^{C}
Mean	6.224^{A}	5.041^{B}	Total mean (5.633)

a-Three replicates were used for each treatment.
b-Different letters refer to the presence of significant differences between treatments at P≤0.05, according to Duncan-test.

Table 2 elucidated that the mean concentration of Fe in uninfected catfish (9.526 µg/gm fresh weight of catfish organs) was higher than that found in infected catfish (4.954 µg/gm). As for body organs, the mean concentration of Fe in liver tissues (10.761 µg/gm) was higher than that in gills (7.300 µg/gm) and muscles (3.660 µg/gm).

TABLE 2: MEAN CONCENTRATION OF FERROUS (Fe) µg/gm FRESH WEIGHT OF CATFISH BODY ORGANS

Body Organ	Mean conc. of Fe µg/gm fresh weight of catfish organ		
	Uninfected fish	Infected fish	Mean
Liver	13.653^{A}	7.870^{C}	10.761^{A}
Gills	10.090^{B}	4.510^{D}	7.300^{B}
Muscles	4.836^{D}	2.483^{E}	3.660^{C}
Mean	9.526^{A}	4.954^{B}	Total mean (7.240)

a-Three replicates were used for each treatment.
b-Different letters refer to presence of significant differences between treatments at P≤0.05, according to Duncan-test.

Table 3 elucidated that the mean concentration of Mn in uninfected catfish (8.538 µg/gm fresh weight of catfish organs) was higher than that found in infected catfish (7.255µg/gm) with significant differences at P≤0.05. Furthermore, the mean concentration of Mn in liver tissues (13.985 µg/gm) was higher than that in gills (9.136 µg/gm) and muscles (0.570 µg/gm).

TABLE 3: MEAN CONCENTRATION OF MANGANESE (Mn) µg/gm FRESH WEIGHT OF CATFISH BODY ORGANS

Body Organ	Mean conc. of Mn µg/gm fresh weight of catfish organ		
	Uninfected fish	Infected fish	Mean
Liver	16.733^{A}	11.236^{B}	13.985^{A}
Gills	7.840^{C}	10.433^{B}	9.136^{B}
Muscles	1.043^{D}	0.096^{D}	0.570^{C}
Mean	8.538^{A}	7.255^{B}	Total mean (7.897)

a-Three replicates were used for each treatment.
b-Different letters refer to presence of significant differences between treatments at P≤0.05, according to Duncan-test.

From **Table 1, 2** and **3** we can conclude that total mean concentration of Mn in different body tissues of the catfish (7.897 µg/gm) was higher than total mean concentration of Fe (7.240 µg/gm). Cr total concentration in catfish organ was the less (5.633 µg/gm).

Variation in concentrations of heavy metals in tissues of aquatic organisms is affected with the concentration of such metals in the surrounding waters and sediments, which are in turn affected by Geochemical seasonal variations and the type of exchanged pollutants to the water stream. According to Iraqi river safekeeping authority (2005), determinatives for Cr, Fe and Mn concentration were 0.05; 0.3 and 0.1 mg/L; respectively [25]. Al-Obaidy *et al.*, (2014) [25] reviled that concentration of Cr and Fe in Tigris river waters in middle Iraq were found to be higher than authorized Iraqi determinatives, especially at wintertime, whereas Mn concentration was lesser than authorized Iraqi determinatives. Khwedim *et al.* (2009) [26] illustrated that the relatively high concentration of Cr in Iraqi lands is due to the effect of leftover sedimentary plains those come from North and Eastern North of Iraqi lands. Which are rich with chromium metal in the form of Cr_2O_4. As for Fe, it is plentiful in igneous rock and sedimentary rock in the form of Fe_2O_3 and $Fe(OH)_2$. Both metals (Fe and Cr) are transferred from soil to Tigris stream *via* erosion and weathering [26]. Mansoor and Said (2018) [27] indicated to the relatively high concentration of VI chromium in the liver, gill, and muscles of the freshwater fish, genus Barbus found in Tigris river in Baghdad-Iraq. Al-Taee (2004) [28] referred to the high activity of Mn compounds in the acidic medium, and that abundance of carbonate and bicarbonate in Tigris stream middle and South Iraq are lessening Mn activity.

Fig. 1 elucidated that mean concentration of the three heavy metals (Cr, Fe, and Mn) were lesser in the body organs of infected fish than uninfected fish; and that liver got the 1st grade as targeted organ for the three heavy metals accumulation, gills and muscles came at 2nd and 3rd grade respectively.

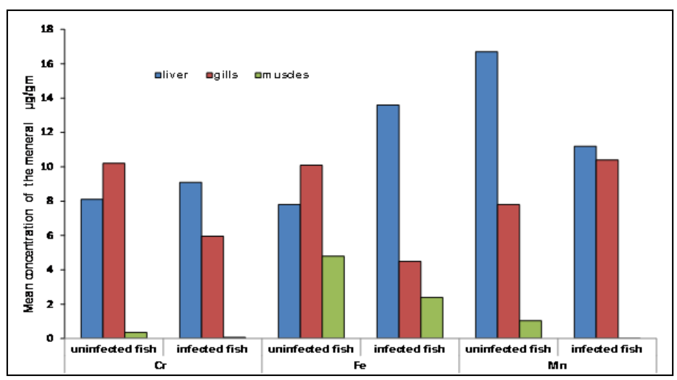

FIG. 1: COMPARISON BETWEEN MEAN CONCENTRATION OF Cr, Fe AND Mn µg/gm OF LIVER, GILLS AND MUSCLES TISSUES OF INFECTED AND UNINFECTED CATFISH

Our results consisted of those of [11, 29, 30], who concluded that heavy metals are accumulated relatively in high concentrations in fish livers and the gills at the second grade compared with low accumulation in fish muscles. Karadede *et al.* (2004) [31] indicated to the relatively high accumulated amounts of the metals Mo, Co, Ni, Zn and Cu: in liver at the highest level, gills at the 2nd level and relatively low accumulation means in muscular tissues of *Liza abu* and *Silurus triostegus* fish those inhabit Ataturk leak in Turkey. It is of Luckiness that muscles are the main part of fish those consumed by man. Accordingly, studies of heavy metals accumulation were focused on fish muscles by some researchers [29, 32, 33] as a step for checking the validity of fish meat for human consumption. Tielen *et al*., (2004) [34] reported that accumulation of some heavy metals in the liver of freshwater fish was relatively higher than gills, both in infected and non-infected fish with intestinal helminths.

On the other hand, the results in the present research were compatible with those of [6, 35, 36], who reported that concentrations of heavy metals in tissues of the parasitized fish were lesser than their concentration in the non-parasitized fish of the same species. Tapeworms have no digestive system and thus gain all necessary nutrients including heavy metals from their host intestine. This may explain why the concentration of heavy metals in host tissues would be lessened. Heavy metals, particularly those belonging to bivalent group, have the ability to cross over gills epithelial lining toward bloodstream, then bind with specific binding proteins like albumin and metallothionein. Such proteins are found in different body tissues, like liver and kidneys and have the ability to form organometallic complex as a step for such metals elimination [37].

Liver is an important location for heavy metals bioaccumulation because it is a location for metal-binding proteins synthesis, detoxification and also has a specific position in circulatory and digestive systems simultaneously [38]. Moreover, gills were also showed high accumulation means of the three heavy metals (Cr, Fe, and Mn) in the catfish, may because they are active site for osmotic- and ionic regulation in fish body, those enable gills to absorb the metals those dissolved in surrounding water [39, 40]. They have wide surface area that permits active gas exchange, and thus allow large amounts of the dissolved metals to access through gills [41], some metals could also be absorbed on the mucous membrane of the gill plates [31]. As for the relatively low concentration of Cr, Fe, and Mn in muscles, it may due to the low affinity of fish muscular proteins to be combined with such metals, or to the low activity of metabolic processes, those lead to bioaccumulation of heavy metals in fish muscular tissues [42]. Baharoma and Ishaka (2015) [43] assumed heavy metals concentrations in five types of freshwater fish, from Galas River- Malaysia. They indicated to the low concentrations of these metals (Zn, Mn, Pb, Ni, and Cu) in muscle tissues correlated to the permissible limit set by Food Act 2003 [43].

Table 4 elucidated that the concentration of Fe in *P. armata* tissues (12.760 µg/gm) was higher than Mn concentration (9.713 µg/gm) and that of Cr (4.903 µg/gm).

TABLE 4: MEAN CONCENTRATION OF Cr, Fe AND Mn µg/gm FRESH WEIGHT OF *P. ARMATA* TISSUES

Heavy metal	Mean concentration µg/gm. fresh weight. of *P. armata* tissues ± SE
Cr	4.903 ± 0.870
Fe	12.760 ± 0.593
Mn	9.713 ± 0.962

Large invertebrates and endoparasites were used by some researchers to evaluate water pollution and alterations in In geophysical factors of the aquatic ambiance [44, 45]. Other researchers were studying environmental health and anthropogenic impact in particular regions using different parasites as bioindicators [7, 36, 46].

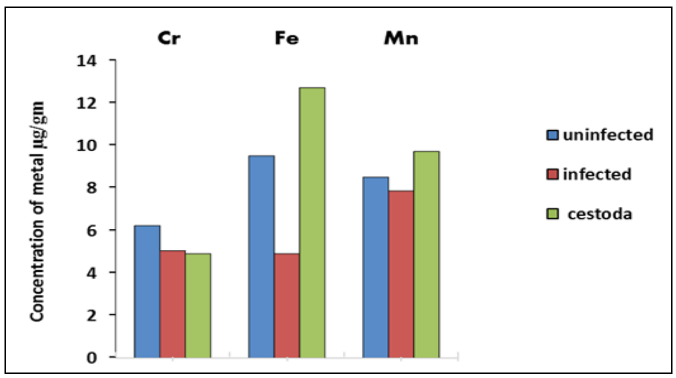

FIG. 2: COMPARISON BETWEEN MEAN CONCENTRATIONS OF Cr, Fe AND Mn (µg/gm) IN BODY ORGANS OF UNINFECTED, INFECTED CATFISH AND *P. ARMATA* CESTODA TISSUES

Fig. 2 illustrated the comparison between the mean concentration of Cr, Fe, and Mn in uninfected, infected catfish tissues and *P. armata* tissues. It has appeared that men concentration of Fe and Mn is higher in tapeworm tissues than in infected fish. Cr concentration in tapeworm tissues was lesser than that in infected and uninfected catfish.

Generally, previous results may be referred to that presence of the intestinal tapeworm may lessen accumulation of the three heavy metals in catfish tissues, since that concentrations of the three metals were lesser in the infected than in uninfected fish. This result is compatible with that of [5, 7, 47], who revealed that concentrations of heavy metals in tissues of the intestinal helminths were higher than the concentration of these metals in their host fish and that Fe accumulated in the tapeworm tissues in higher concentration than other metals.

Access of metals to fish mouth during nourishment leads to accumulation of heavy metals in the intestinal tissues and lumen; the state that may lead to additional accumulation of metals in the tapeworm tissues at higher rates than in fish tissues. Such case may due to competition between the parasitic helminths and its fish host on food and other materials including heavy metals [48]. Furthermore, additional fat-soluble pollutant metals can inter worm tissues accompanied with the fatty acids that absorbed from host intestine [49]. As for the higher concentration of chromium in tissues of catfish compared with that in *P. armata* tissues, it may due to the entry of chromium through fish gills rather than GIT. Bergey *et al*. (2003) [50] stated that when heavy metals interning fish body via gills rather than intestine, it is often accumulated at higher concentration in fish tissues than in intestinal helminths those parasites the fish.

Ultimately, our results proved that studying bioaccumulation of heavy metals in endoparasites (particularly tapeworms) was a successful model that could be used as bio-indicators for water pollution with heavy metals and for periodical evaluation of aqueous ecosystems state in Iraqi lands, this opinion is to agree with those of [46]. Tellez and Merchant (2015) [51] suggest that parasites, particularly intestinal trematodes, are superior bio magnifiers of some heavy metals, particularly As, Cu, Se, and Zn. Hassan *et al*., 2016 [52] concurred that parasitic nematodes of the kosher fish *Epinephelus summana* caused significant decreases in some heavy metals in fish tissues including lead iron and calcium compared to uninfected fish. Estimation heavy metals that accumulation in endo and ectoparasites, even larvae or adult stages are reflected heavy metals pollution in a particular aqueous culture.

Furthermore, it may refer to the exposure period to such metal [45]. Some researchers were considering that Fish, large invertebrates and endo-parasites are valuable tool for the established guidelines for effective environmental management, and could be used as bio-indicators for evaluating environmental health, environmental alters, and the impact of anthropogenic activities) [7, 12, 18, 36].

CONCLUSION: Concentrations of the three studied heavy metals: Cr, Fe, and Mn were higher in the tapeworm tissues *P. armata* than in infected catfish tissues, means that the three heavy metals were successfully bioaccumulated in the worm tissues with variable averages. The concentration of the three heavy metals in different tissues of the infected catfish was lesser than in the uninfected fish, referred to that presence of the parasitic tapeworm has an apparent role in the lessening of heavy metals accumulation in the tissues of the parasitized fish.

Determination of heavy metals concentration in a riverbed, riverside and river water, and the resident organisms (some plants and animals species) considering seasonal variation is necessary to give an accurate idea about bioaccumulation, bio-concentration, and biomagnification of certain heavy metals and maybe the pollution level in a specific river.

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CONFLICTS OF INTEREST: Nil

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