Determination of trace metals in some organs of *Carassius carassius* (Linnaeus, 1758) fish and histopathological change in the Babil drainage network, Iraq

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Received: May 2022 Accepted: August 2022

Abstract

This study was conducted to estimate the differences in the accumulation of heavy metals (titanium, barium, lithium) among the body organ of the *Carassius carassius* fish (liver, gills, kidneys). caught from the Babil drainage network collected on January 10, 2022, at a rate of 30 samples fish are found in abundance in the drainage network due to the Brackish water's high salinity compared to river water, and the fish were of an average length (10-12) cm. The results showed that the concentrations of barium were highest in the gills, followed by its content in the liver and then the kidney in the same context, the concentration of titanium in the kidney was the lowest, and it had no significant difference from its content in the liver, although there was a numerical difference between them, and the highest concentration of titanium was in the gills reaching (33.7, 41.33, 135) ppm, respectively.

On the other hand, the highest concentration of lithium was in the kidney, then gills, and liver, respectively, which had no significant difference between them. The gills damaged lesion was characterized by showing congestion of central venous sinus vessels and extensive sloughing of primary and secondary lamellae, with inflammatory cells infiltrations The macroscopic examination for kidney showed the atrophy of glomerular tufts and infiltration of melanomacrophages also excessive enlargement of thyroid follicles that filled with colloid materials .Results of microscopically examination of a kidney showing marked depletion of hemopoietic tissues which shows a histological section of the liver of wild *Carassius carassius* during the study period, showed the necrosis with extensive mononuclear cells infiltration.

Keywords: *Carassius carassius*, Babil drainage network, *Crucian carp*, Barium (Ba), Lithium (Li), Titanium (Ti), Heavy metal

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Introduction

Pollution of water bodies around the world has increased several times due to the presence of both organic and inorganic pollutants and is currently an indisputable issue. Considerable consideration has been given to chemical pollutants (Alshkarchy *et. al.*, 2021; Mansour and Sidky, 2002). Among these pollutants, trace elements are the most dangerous because, they are toxic and bio accumulative in nature, and in the long run, They pose a threat to human health who consume these trace elements by eating fish and other foods containing these elements consume these minerals (Dar *et. al.*, 2013). Although heavy metals are natural components of the aquatic environment, their levels have increased due to the sources Humanity.

Aquatic ecosystems are enriched with heavy metals due to agricultural drainage and industrial wastewater (Jeelani and Shah, 2006). Accelerated development activities in and around water bodies around the world are responsible for declining water quality (Sujatha *et. al.*, 2001; Rather *et. al.*, 2019). Effects of human activities on water require continuous monitoring in order to discover and characterize the causes and sources of these water pollutants. Among the monitoring tools are aquatic organisms (insects, fish, frogs, plants) which are known as potential biomarkers that reveal the presence of contaminants in water (Jia *et. al.*, 2017). When tracking long-term changes in a specific water body such as a river, lake, and pond, fish are considered reliable biomarkers for long-term monitoring of pollution, as reported by several researchers over the years (Benejam Vidal, 2008). The high percentage of heavy metals in fish that use for human consumption, but different waste must be disposed of in a safe way to control the accumulation of heavy metals in the future to get rid of the diseases that cause them to consumers (Salam *et. al.*, 2019; Al-Musawi *et. al.*, 2021)

In the present study, carp crucians (*Carassius carassius)*, omnivorous and prevalent freshwater fish, were chosen as experimental subjects. The reproductive ability of carp carp crucians is exceptionally high, and they are often consumed by humans and are thus closely related to them (Hu *et. al.*, 2022).

Materials and methods

Description of the study area

Drainage network in the village of Al-Sadah area, north of the Babylon city and it is located within the coordinates N32˚44'19.169" E44˚16'5.19". The river is about 100 m wide and 6 m deep.

Sample collection

Samples of 30 wild fish *Carassius carassius* were obtained using a cast net. The caught fish were placed in plastic containers of 50 liters and filled with river water. For a period of three hours, they were transported under refrigeration to the fish pathology laboratory at the College of Veterinary Medicine, in Al-Qasim green University, tissue extraction (gill, kidney, liver) to estimate Barium (Ba) Lithium (Li) Titanium (Ti), fish that appeared in good health were selected with an average length of 12-14 cm. Some environmental properties of water were field-measured using a multi-meter and included water temperature (c), pH, salinity (mg /L), and O_2 (mg /L)

Trace metals extraction

The process (ROPME, 1982) was used to digest the aforementioned fish samples using the digestion procedure until the concentration of (Ba, Li, and Ti) measured Metals with ICP-MAS inductively coupled plasma mass spectrometry (Monroy *et. al.*, 2014).

Histological examination

The fishes were sacrificed at the laboratory after being caught, , then samples were taken, which included (liver, kidney, and gills) and fixed with formalin solution at a concentration of 10% and it was replaced after 24 hours with formalin solution with the same concentration as the previous (10%) and the preparation of slides was completed. Histologically according to the method of (Bancroft and Stevens, 1975) and stained with hematoxylin eosin double stain, the microscopic slides were examined using an optical microscope, on - Olympus type, and the microscopic slides were photographed using a light microscope equipped with a digital imaging camera with different magnification powers.

Statistical analysis

The experiment was designed according
to the completely randomized design (CRD) and the use of the ready statistical analysis system in analyzing the effect of the parameters on the studied traits. The significant differences between the averages of the studied traits were tested according to Duncan's multiple range test. At a significant level (Duncan, 1955).

Results

Chemical and physical factors

Water temperature level was (16.3°C) during the winter season 2022 as seen in Table 1. pH values during the study period was (6.9), and salinity value was reported during the winter (0.801‰), while the dissolved oxygen was recorded (7.2) mg/L in January 2022, as shown in Table 1.

Table (1) Chemical and physical properties of water in the study site.

Temp °C	16.3
PH	6.9
Salinity‰	0.801
O_2 mg/L	7.2

Concentration of heavy metals in fish organ

Barium (Ba): The results showed significant differences for the Ba between kidney, liver and gills for all samples, as the highest concentrations was recorded in the gills and the concentrations were (54.33±2.96) ppm, while they were in the kidney and liver (17.17±1.9 and 16.17±1.9) ppm respectively As shown in Table 2.

Lithium (Li): results were as follows, the highest value in the kidney, when it reached 15.6±0.2 ppm, no significant difference was observed between gills and liver, as it was recorded (11.667±0.667and 9.9±0.666) ppm respectively, As shown in Table 2.

Titanium (Ti): The results showed significant differences of Titanium element between all samples, the concentrations were (135±17.21, 41.33±1.855 and 33.7±1.86) ppm for each of the, gills liver and kidney, respectively. The gills had the highest accumulation, then the liver, and then the kidney. As shown in Table 2.

Table 2: Concentrations of Ba , Li and Ti in *Carassius carassius* Tissue from the study areas ppm.

Heavy metal		Organ		
		Kidney	Liver	Gills
Ba	Mean	17.17 bc	16.17 b	54.33 a
	SE	±1.9	1.9±	2.96±
Li	Mean	15.6 a	9.9 bc	11.667 b
	SE	0.2±	0.666±	±0.667
Ti	Mean	33.7 bc	41.33 b	135 a
	SE	1.86±	1.855±	17.21±

Means with the same letter in same metal are not significantly different.

Histological changes

The result of histopathological examination in gills were congestion of central venous sinus vessels and extensive sloughing of primary and secondary lamellae with inflammatory cells infiltrations as shown in Figure 1, while in the kideys the result showed atrophy of glomerular tufts , infiltration of melanomacrophages , also excessive enlargement of thyroid follicles that filled with colloid materials as shown in Figure 2 also showed marked depletion of hemopoietic tissues Figure 3. Microscopic examination of the liver sections were showed necrosis with extensive mononuclear cells infiltration as in Figure 4.

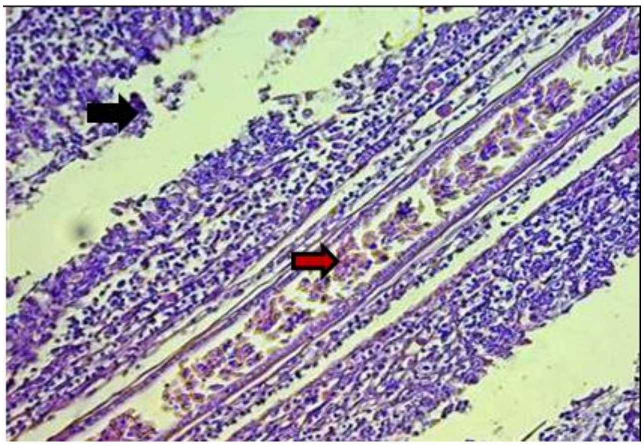

Figure 1: Histopathological section of fish gill showing congestion of central venous sinus vessels (red arrow), and extensive sloughing of primary and secondary lamellaewith inflammatory cells infiltrations (black arrow). H&E stain, 200X.

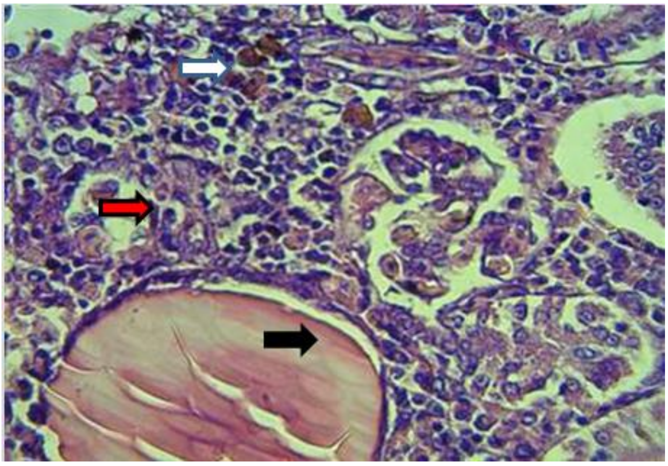

Figure 2: Histopathological section of fish kidney showing atrophy of glomerular tufts (red arrow), infiltration of melanomacrophages (white arrow) also excessive enlargement of thyroid follicles that filled with colloid materials (black arrow). H&E stain, 400X.

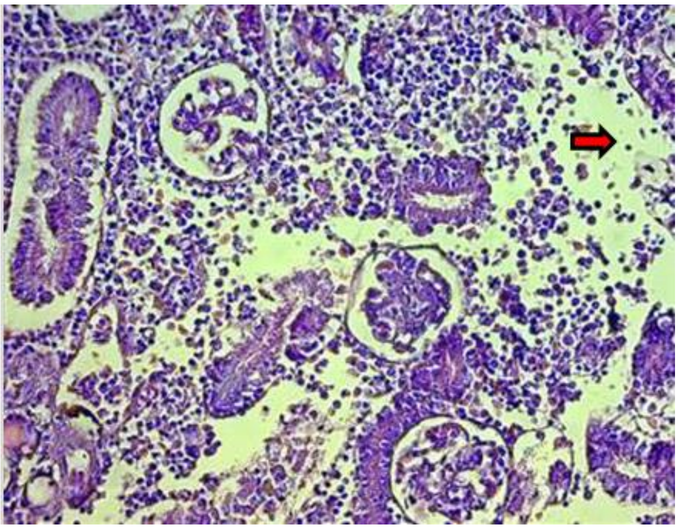

Figure 3: Histopathological section of fish kidney showing marked depletion of hemopoietic tissues (red arrow). H&E stain, 200X .

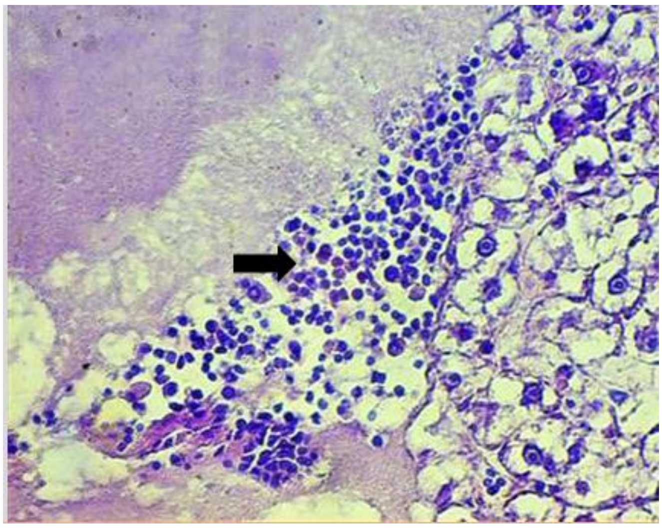

Figure 4: Histopathological section of fish liver showing necrosis with extensive mononuclear cells infiltration (red arrow), H&E stain, 400X.

Discussion

Heavy metals in fish

The results showed that the accumulation of metals in the gills, kidney and liver differed according to the mineral studied .The reason for this is that differences in the accumulation of heavy metals in fish tissues are affected by some factors depending on the metabolism of the element by the fish, it is distributed to different tissues depending on biological needs (Alshkarchy *et. al.,* 2021)

The results of the statistical analysis showed that the highest concentrations of Ba and Ti was recorded in the gills were (54.33,135) ppm respectively ,This may be due to gills are the main pathway for the ion exchange of elements from the water because they have very large surface areas that facilitate the rapid diffusion of toxic elements (Alm-Eldeen *et. al.,* 2018) . A multifunctional device, all breathing and ionic and osmotic regulation, as it is the most important areas of ion exchange between the environment and the organism, and the gills are the main site for the absorption of pollutants and chemicals. In the same context, local studies conducted on the Euphrates River agreed to measure the concentration of heavy metals in the tissues of carp fish, including (Al-Khafaji *et. al.,* 2011), which conducted a measurement of the concentration of some heavy metals, a study on the Euphrates River in the city of Nasiriyah in the sediments and various organs of common carp fish. The results obtained were a high concentration of heavy metals in the gills due to the presence of chloride cells that facilitate the accumulation of heavy metals in them.

Mustafa *et al*. (2020) stated in a study he conducted on the Tigris River to study the level of accumulation of a number of heavy metals in common carp fish that the concentration of heavy metals was high in the gills and attributed the reason for this to the fact that the gills in freshwater fish are the main entry point any heavy elements dissolved. Or, the reason for this may be that the accumulation of heavy metals in general is more evident in the gills and liver than in the muscles, as the active metabolic organs accumulate higher amounts of heavy metals, and this has been confirmed by several international studies, including them (Tekin-Özan & Kir, 2008).

The results showed that the accumulation of Ba and Ti in the, liver and kidney has no significant deference between them Although, the accumulation was numerically higher in the liver than in the kidney . In the same context, previous research conducted on juvenile fish (rainbow trout, *O. mykiss*) indicated that the accumulation and absorption of titanium is limited by internal organs when exposed to certain concentrations in water or through food (Federici *et. al.,* 2007; Ramsden *et. al.,* 2013). The results of (Chen *et. al.,* 2011) showed the accumulation of trace elements after exposing zebrafish for a period of 6 months.

The exposure of fish to high concentrations for a long period led to the difference in the accumulation of trace elements among the studied organs, and this was supported by the tissue sections. It is worth noting that (Federici *et. al.,* 2007) noted some significant edema and epithelial damage in the trout gill. On the other hand, Ramsden *et al*. (2013) stated, through histology, there was no evidence of acute edema, epithelial elevation or reactive hypertrophy in the gills, perhaps the reason for this is that the experiment period was short and the lifespan of the fish was small compared to the fish in the current study .We conclude from this that the accumulation of trace elements on the body organs in direct contact with water (such as the skin and gills) is higher than the rest of the organs and causes mild respiratory distress that led to pathological changes in the study (Ramsden *et. al.,* 2013). As a preventive measure as a result of oxidative damage, as well as a lack of any electrolyte depletion from tissues.

Conclusions

In the conclusion of the research, we can conclude that the deposition the element barium and titanium in Carassius *carassius* fish It is higher in the gills, then the liver and kidneys are lower. This may depend on the type of fish, while lithium is mainly deposited in the kidneys, and there was no difference in its deposition between gills and liver.

Acknowledgment

The authors are indebted to the College of Veterinary Medicine resources, Al-Qasim Green University for the support of this project, and thanks for workers on the Atomic lab.

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