HISTOPATHOLOGYANDLEVELOF BIOACCUMULATION OFSOME HEAVY METALS IN FISH, *CARASOBARBUSLUTEUS* AND *CYPRINUSCARPIO*TISSUES CAUGHTFROM TIGRIS RIVER, BAGHDAD

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ABSTRACT

This study was undertaken to detect the histopathology and level of bioaccumulation of lead and cadmium in water and in some freshwater fish (*Carasobarbus luteus* and *Cyprinus carpio*) tissues caught from Tigers River. A total of 100 water samples and 100 fish specimens (kidney, gills, liver and muscle) were collected from three sites of Tigris River. These samples were used to detect Pb and Cd via Atomic Absorption Spectrophotometry.Results exhibited high concentrations of Pb and Cd in water samples (>0.03 for both metals) and insome organs of the selected fish. It was observed that the levels of Pb and Cd accumulated in most organs (kidneys being most influenced) followed by gills, livers and muscles at three sites. The levels of these heavy metals were much above the maximum acceptable limit recommended by FAO and WHO. Histopathology was also conducted where heavy damages were noticed in both livers and gills in both fish species. The current study present data about increasing pollution in the Tigris River and it approves that it is having strong impact on fish health and on human beings.

Keywords: cadmium,kidney, liver, lead, muscle, specimens.

مجلة العلوم الزراعية العراقية -2020 :51(2):698-704 مصطفى وآخرون

التشريح المرضي والمستوى التراكمي لبعض المعادن الثقيلة في انسجة الأسماك (*Carasobarbus luteus* و *Cyprinus carpio*) المصيدة من نهر دجلة، بغداد

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المستخلص

اجري البحث لدراسة التشريح المرضي والمستوى التراكمي للرصاص والكادميوم في الماء وفي بعض انسجة أسماك المياه العذبة (*Carasobarbus luteus* و *Cyprinus carpio*) التي تم صيدها من نهر دجلة. تم جمع عينات من المياه والأسماك (الكلى والخياشيم والكبد والعضلات) من ثلاثة مواقع لنهر دجلة. واستخدمت هذه العينات للكشف عن Pb و Cd عبر المقياس الطيفي للامتصاص الذري.اظهرت نتائج الدراسة معدلات عالية من Pb و Cdفي انسجة عينات كلاالنوعين من الاسماك. وقد لوحظ أن مستويات Pb و Cd المتراكمة في معظم الأعضاء (الكلى كانت الأكثر تأثراً) تليها الخياشيم والكبد والعضلات في ثلاثة المواقع. كانت مستويات هذه المعادن الثقيلة أعلى بكثير من الحد الاعلى المسموح به التي أوصت به منظمة الصحة العالمية ومنظمة الأغذية والزراعة. أجري التشريح المرضي أيضًا حيث لوحظت افات نسجية في كلاً من الكبد والخياشيم في كلا النوعين من الاسماك اظهرت الدراسة الحالية نتائج حول التلوث المتزايد في نهر دجلة والذي يدل على أنه له تأثير قوي على صحةكل من الأسماك والانسان.

الكلمات المفتاحية: الكادميوم، الكلية، الكبد،الرصاص، العضلات، العينات

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INTRODUCTION

The development of modern industrialization and rapid technology are responsible for aquatic ecosystem pollution. Discharged waste from anthropogenic activitiesinto the water environmenthas an influence on thefauna and flora (11, 14). The pollution of the Tigris River with high levels of hazardous materials and chemicals has long been a very serious environmental issue and a major public health problem in Iraq (3, 6, 17, 30), principally due to domestic, agriculture, industrial activitiesand other byproducts (16, 19). Recently, the Tigris River has become heavily contaminated with various types of pollutants and has reached an alarming rate which highly affecting aquatic species and human life. Heavy metals represent the most dangerous type of pollutants as it has been well known that heavy metals, such as chromium, cadmium, copper and lead areextremely toxic to human tissues (15). Accumulation of heavy metals are known to adversely affect the liver, gills, kidney, muscle and other tissues of fish (10, 18, 28) which can be visualized via histopathology (1, 2).Studies up to now have been focused on demonstrating the quality of the River water but there is few data about impact of toxicity of heavy metalson fish in particular the histopathology. Therefore, this study is useful to create awareness so that some preventive measurement must be employed by the Government to protect the health of population and environment. Hence, this study was undertaken to detect the histopathology and level of bioaccumulation of heavy metals (Pb and Cd) in water and fish tissues caught from Tigers River, Baghdad.

MATERIALS AND METHODS

Study area

The sampling sites from Tigris River were located in southern of Baghdad city, included three sites along Tigris River. The first site was selected as a control or reference site due to its remoteness from any human and industrial activities. This site was lies close to water sewage of the Karkh Directorate. The second site was located nearby confluence of the Diyala River with Tigris River. The third site was situated nearby Ibn –Khateeb Hospital (Figure.1).

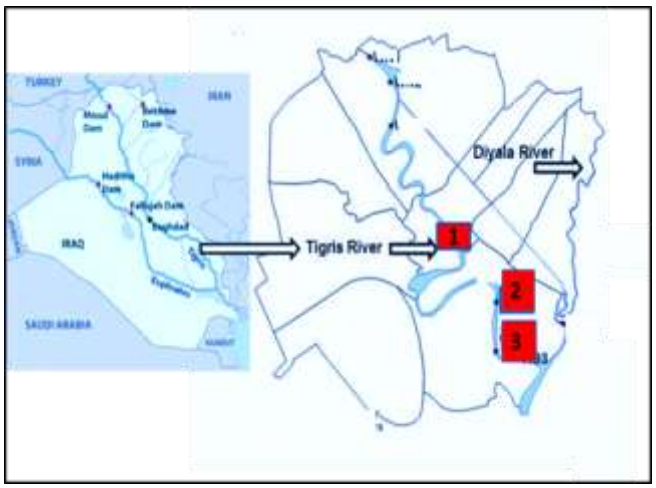

Figure1. Map showing three sampling sites from Tigris River.

Collection of fish and water specimens

Fishes (100 sample) were collected during March to June 2018 (average weight 150-200 g; length 20 cm) were dissected and organs (liver, gills, kidney and muscle) were removed. Also, 100 water samples were collected from three sites of Tigris River. Water samples were kept for the heavy metals analysis according to standard techniques of APHA, AWWA and WEF (4).Concurrently, fish (*Carasobarbus luteus* and *Cyprinus carpio*) specimens also were caught with the assistance of local fisherman Physico-chemical properties of the water (dissolved Oxygen, pH, temperature and salinity) were registered at the site itself.

Samples analysis

Digestion of the tissue was conducted to estimate the level of bioaccumulation of lead and cadmium (Pb and Cd) according to the standard method suggested by Mustafa *et al.*(27). Heavy metals (Pb and Cd) were analyzed using Atomic Absorption Spectrophotometer and results are expressed mg L^{-1} (for water samples) and in µg g^{-1} (for tissue specimens).

Histopathology

The live fishes (soon after catching) were dissected out and the organs (liver and gills) were fixed in formaldehyde solution at 10% for 48 h. Tissue processing, blocks

preparation and staining of the sections using Hematoxylin and Eosin stain were conducted as described by Mustafa et al. (26). The prepared slides (6 µm sections) were examined under the microscope (Optica) at 400X and observed for histopathological alterations.

Statistical analysis

Data were analyzed as mean and standard deviation (SD) using One-way analysis of variance (ANOVA) followed by Duncan's new Multiple Range Test (MRT) to compare the difference amongst the means. *P*-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Physico-chemical parameters of water

The physico-chemical properties for the three sites of Tigris River water are dissolved oxygen level was 4.2-4.7 mg L^{-1}, pH was 7.24-8.12, the temperature ranged between 28.6-23.2°C. Salinity was 0.39-0.75 g L^{-1}.

Heavy metals analyses in water and fish samples

Lead (Pb) and Cadmium (Cd) level (µg L^{-1}) in water samples are present in Table 1. These levels were above the recommended values set by WHO (33). Bioaccumulation of Pb and Cd (µg g^{-1} wet weight) in different tissues of *C.luteus* and *C. carpio* was determined and is shows in Table 2 and 3. In *C. luteus* Pb at site 2 recorded the highest value in kidney (87.21 µg g^{-1}) followed by gills (57.22 µg g^{-1}), liver (52.41 µg g^{-1}) and muscle (36.17 µg g^{-1}). This site was significantly different ($P<0.05$) compared to site 1 and 3. Level of Cd recorded the highest values at site 2 in kidney (3.94 µg g^{-1}) followed by liver (3.52 µg g^{-1}), gills (2.54 µg g^{-1}) and muscle (2.44 µg g^{-1}). Site 2 showed significant differences compared to site 1 but it was not significant ($P>0.05$) compared to site 3. In *C. carpio* the pattern detected was similar to *C. luteus*(Table 3). At site 2 Pb accumulated the most [kidney (56.80 µg g^{-1}), liver (62.20 µg g^{-1}), gills (44.30 µg g^{-1}) and muscle (50.70 µg g^{-1})]. This site was significantly different ($P<0.05$) relative to site 1 and 3. For the Cd kidney recorded the highest value (3.83 µg g^{-1}) followed by gills (3.53 µg g^{-1}), gills (2.98 µg g^{-1}) and muscle (2.43 µg g^{-1}). Kidney was the most influenced organ in both above mentioned fish species. The mean concentrations for the two metals (Pb and Cd) in the kidneys, gills, livers, and muscles of both fish samples (*C. luteus* and *C. carpio*) at three locations were much higher maximum acceptable limit of heavy metals in freshwater fish according to FAO (13)and to WHO (33). The results of this study showed that the highest heavy metal levels were found in the kidneys, gills and livers of both sampled fishes. The reasons for such increased concentrations of heavy metals in the both sampled fishes particularly at the second site were due to the industrial wastes discharged from the factories without proper treatment located along the Tigris River in Baghdad. Several researches have been showed similar resultsin Iraq (6, 7, 9, 22, 32). The high concentration of heavy metals in the gills of *C. luteus* and *C. carpio* was due to the fact that the gills in freshwater fishes are the major entry point for any dissolved heavy metals (29). These results are comparable to other studies that revealed the levels of heavy metals in the kidneys, gills and livers of fishes was higher than the bioaccumulation of heavy metals in the muscles of fishes (20, 25).

Table1. Levels of Pb and Cd (µg L^{-1}) dissolved in water at three sites of Tigris River

Site*	Pb	Cd
1	0.06±0.002b	0.03±0.00a
2	1.24±0.006a	0.06±0.001a
3	1.01±0.003a	0.04±0.001a

Data are means ±SD. Different letters vertically indicated significantly different at $P\leq0.05$

Site 1 was located close to water sewage of the Karkh Directorate.

Site 2 was located nearby confluence of the Diyala River with Tigris River

Site 3 was situated nearby Ibn –Khateeb Hospital

Table 2. Levels of bioaccumulation of heavy metals Pb and Cd (µg g^{-1}) in *C.luteus* in various tissues at three sites

Levels of bioaccumulation of Pb (µg g^{-1}) in different tissues at three sites				
site	kidney	gills	liver	muscle
1	21.13±8.22c	29.24±06.44c	21.25±4.12b	11.41±3.71c
2	87.21±11.43a	57.22±13.22a	52.41±9.21a	36.17±7.51a
3	69.24±11.46b	32.42±09.72b	42.19±06.18a	22.16±04.23b
Levels of bioaccumulation of Cd (µg g^{-1}) in different tissues at three sites				
1	1.11±0.05c	1.76±0.81b	2.11±0.11b	1.14±0.91c
2	3.94±0.86a	2.54±0.53a	3.52±0.700a	2.44±0.72a
3	2.82±0.91a	2.04±0.54a	3.75±0.40a	2.87±0.92a

Data are means ±SD. Different letters vertically indicated significantly different at *P*≤0.05.

Table 3. Levels of bioaccumulation of heavy metals Pb and Cd (µg g^{-1}) in *C.carpio* in various tissues at three sites

Levels of bioaccumulation of Pb (µg g^{-1}) in different tissues at three sites				
site	kidney	gills	Liver	muscle
1	16.42±1.90c	19.10±5.00b	12.64±02.00c	15.20±03.10c
2	56.80±06.00a	44.30±07.00a	62.20±11.20a	50.70±09.00a
3	41.33±09.60b	36.50±09.00a	43.92±06.00b	44.22±08.00a
Levels of bioaccumulation of Cd (µg g^{-1}) in different tissues at three sites				
1	1.57±0.09c	1.98±0.05c	0.62±0.03b	0.12±0.03b
2	3.83±0.87a	3.53±0.85a	2.98±0.20a	2.43±0.40a
3	2.41±0.60b	2.78±0.55b	2.66±0.60a	1.89±0.10a

Data are means ±SD. Different letters vertically indicated significantly different at *P*≤0.05

Histopathology

Marked changes were observed in liver and gills. Industrial effluents induced significant histopathological changes of both fishes. Liver of *C. luteus* showed normal histology of hepatic tissue with some vacuolization of cytoplasm and nuclear pyknosis (Figure 2A&B). The influenced liver of *C. carpio* revealed congestion in blood vessels with necrosis in liver tissue can also be seen (Figure 2 C&D). Similarly, gills morphology also changed markedly due to the impact of heavy metals in the water. Gills of *C. luteus* these revealed epithelial lifting elongated of secondary lamellae (Figure. 3A&B).While, gills of *C. carpio*expressed hyperplasia and complete fusion of epithelial cells, edema and epithelial lifting. Teleost liver is the main target organ for xenobiotics metabolism and hence, commonly cited as the site of parenchymal damage following exposure to different chemical materials (12). Similarly, Radhakrishnan and Hemalatha (31) whom showed marked cytoplasmic vacuolization and dilation of sinusoids. Also, these changes are in agreement with Mustafa *et al.* (28) how showed similar changes in liver of *C. carpio*exposed experimentally to lead. For the gills, most of these changes in both fishes are considered general defense responsefrom the fish to increase barrier between the blood circulation and external environment (21). Similar alterations are observed by Al-Rudainy and Khalel in *C. carpio* (5), Mohamed (24) in *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt. Also, these changes are in line with Mobarak and Sharaf (23) whom noticed similar findings in gills sections of silver sail fin, *Poecilia latipinna* and with Badr *et al.* (8) in gills tissues of Nile Tilapia caught from River Nile Basin, Cairo, Egypt. The current study present data about increasing pollution in the Tigris River and it approves that it is having strong impact on fish health and on human beings as these heavy metals (Pb and Cd) are accumulating in various organs of the selected fish species (i.e., *C. luteus* and *C. carpio*). This is further supported by the abnormalities that occur in two most important organs (livers and gills). Further researches are needed in order to study the effect of these heavy metals on the

reproductive system as it will reveal the decline in the population of species inhabiting the site selected.

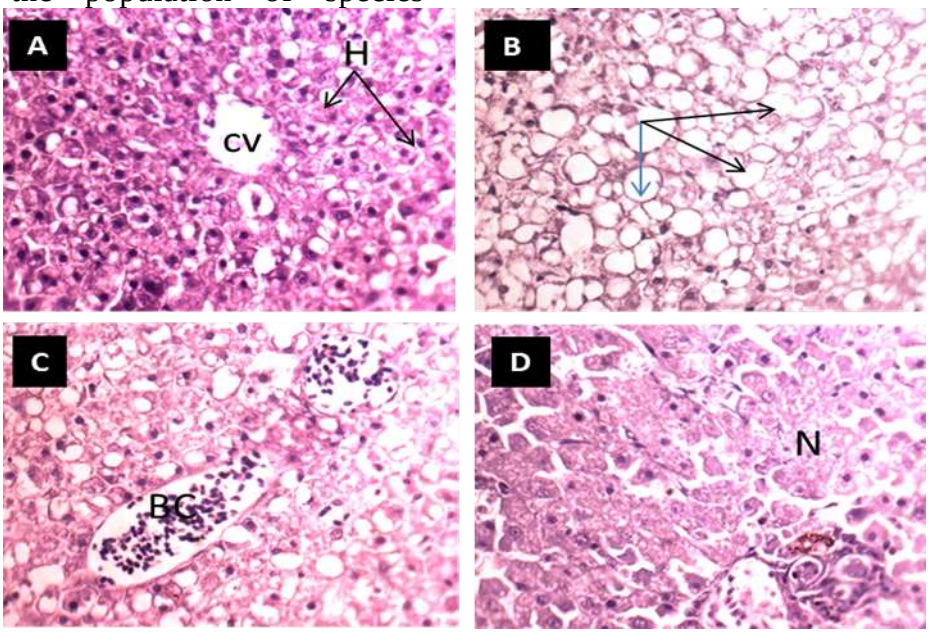

Figure 2: Transverse section of liver from two fishes, (A &B) liver of control C. *luteus* characterized by normal hepatic cells (HC) and presence of central vein (CV) (B) cytoplasmicvacuolization (black arrow) with Pyknotic Nuclei (blue arrow). (C&D) Liver of *C. carpio* showed blood congestion (BC), Pyknotic Nuclei and Necrosis (N). H&E, (400x).

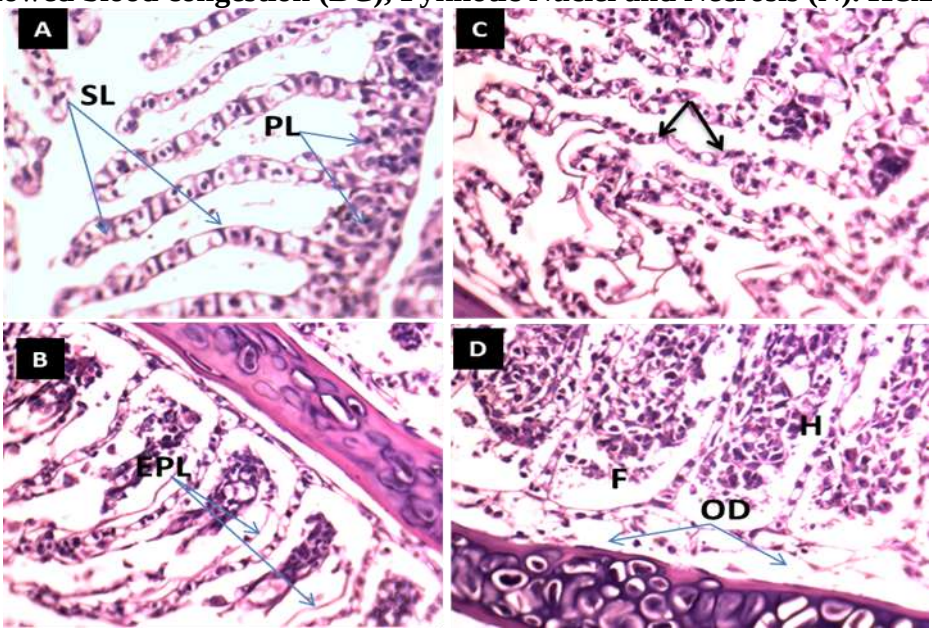

Figure 3: Transverse section of the gills from two fishes, (A&B) gills of *C. striatus* (A) gills of control fish showing normal primary (PL) and secondary lamellae (SL), (B) showing epithelial lifting and elongatedof secondary lamellae. (C&D) gills of *C. carpio* showing epithelial lifting (EPL), edema (OD)and hyperplasia (H) with complete fusion of secondary lamellae (F). H&E.(400x).

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