HISTOPATHOLOGY AND LEVEL OF BIOACCUMULATION OF SOME HEAVY METALS IN FISH, *CARASOBARBUS LUTEUS* AND *CYPRINUS CARPIOTISSUES* CAUGHT FROM 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 in some 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.
INTRODUCTION
The development of modern industrialization and rapid technology are responsible for aquatic ecosystem pollution. Discharged waste from anthropogenic activities into the water environment has an influence on the fauna 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 activities and 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 are extremely 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 metals on 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 Tigris 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).

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. C. carpio at site 2 accumulation 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 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 results in 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).

Table 1. 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≤0.05
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

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

| site | kidney | gills | liver | muscle |
|------|--------|-------|-------|--------|
| 1    | 21.13±8.22c | 29.24±6.44c | 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±9.72b | 42.19±6.18a | 22.16±4.23b |

Levels of bioaccumulation of Cd (µg g⁻¹) in different tissues at three sites

| site | kidney | gills | liver | muscle |
|------|--------|-------|-------|--------|
| 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±0.70a | 2.44±0.72a |
| 3    | 2.82±0.91a | 2.04±0.54a | 3.75±0.40a | 2.87±0.92a |

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

| site | kidney | gills | Liver | muscle |
|------|--------|-------|-------|--------|
| 1    | 16.42±1.90c | 19.10±5.00b | 12.64±2.00c | 15.20±0.310c |
| 2    | 56.80±0.60a | 44.30±0.70a | 62.20±11.20a | 50.70±0.90a |
| 3    | 41.33±0.60b | 36.50±0.90a | 43.92±0.60b | 44.22±0.80a |

Levels of bioaccumulation of Cd (µg g⁻¹) in different tissues at three sites

| site | kidney | gills | Liver | muscle |
|------|--------|-------|-------|--------|
| 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 |

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 2C&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. carpioexpressed 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. carpioexposed experimentally to lead. For the gills, most of these changes in both fishes are considered general defense response from 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) cytoplasmic vacuolization (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 elongated of 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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