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Determination of heavy metals in water of Diyala River and effect on histopathological changes in Tilapia fish

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Abstract

The objective of the present study was to detect histopathological changes in Tilapia fish were obtained from meeting point Tigris River, with the Diyala River in Al-Zaafaraniya area. As well as description clinical signs and behavior of Tilapia fish. The weights ranging from (40-70)gm, mostly females and total length ranging from (15-17)cm. transferred to Ichthyopathology Laboratory of college of Veterinary Medicine in University of Baghdad by cork to conduct the necessary tests in a sterile laboratory conditions. Results of Histopathological changes in gills characterized by Epithelial lifting of secondary lamella, Central Venous Sinus congestion and secondary lamella fusion. Fish behavior was recorded showed abnormalities after exposure to the various degree of contamination such as swimming disorders, the fish tended together at the surface, decrease in respiration rate, fast movement, fish weakness, opened and closed in operculum festally, this study referred to importance of environmental pollution and its direct relationship on fish from its influence histological on gill fish which is considered the most important organ of the fish, it is responsible for the process of breathing and gaseous exchange in the fish.

Keywords: Heavy metals, pollution, histopathology, Tilapia fish

Introduction

In the last years appear increased in fish production in the Arabian world and in the Iraq especially lead to increase pollution and because excessive culture for fish helped in distribution many of contaminant and that's help in disease distribution and histopathological changes in fish ^[1].

Fish exchange gases using gills on either side of the throat. Gills are tissues which consist of threadlike protein structures called gill lamellae. These lamellae have many functions including the transfer of ions and water, as well as the exchange of oxygen, carbon dioxide, acids and ammonia ^[2]. Each lamella contains a capillary network that supports a large surface area for exchanging oxygen and carbon dioxide. Fish exchange gases by pulling oxygen-rich water through their mouths and pumping it over their gills. The gills push the oxygen-poor water out through openings in the sides of the pharynx.

The gills are composed of comb-like filaments, the gill lamellae, which help increase their surface area for oxygen exchange ^[3].

The study of organisms as pollutant monitors has several advantage over the chemical analysis of a biotic compartment. Chemical, toxicological and ecological approaches have been studied extensively in assessing impact of heavy metal pollution in aquatic environments. Most of the papers published on organisms as pollution bioindicators have concentrated on invertebrates, mainly molluscs and crustaceans ^[4].

Environmental pollution is a worldwide problem as heavy metals belong to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems ^[5]. Environmental pollution can cause poisoning, diseases and even death for fish.

The objective from this study was explain effect of some environmental contamination (heavy metals) and excessive cultured (crowding for fish) on behavior of fish which found in river and effect on histopathological changes in gill for Tilapia fish.

Materials and Methods

This study was conducted in the College of Veterinary Medicine, University of Baghdad, Ichthyopathology Laboratory. Examined Tilapia fish from meeting point Tigris River with the

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Diyala River in Al-Zaafaraniya area. The weights ranging between (40-70)gm, and (15-17)cm in total length examined fish for six month beginning from November month to April month, examined fish clinically from movement, respiration, food intake, responses to the outer effects, swimming, gill color, amount of mucous and checked gill lamella. Heavy metals extraction from the filtered water of river One liter of water sample was filtered using millipore filter papers (0.45 µm). These filter papers were washed with hydrochloric acid (5%), followed by de-ionized water and dried at 60 °C for 24hr; the filtered water was transferred to a beaker then acidified with 5 ml of concentrated nitric acid.

The sample allowed to cool and transferred to 50 ml volumetric flask, The solution was kept in clean polyethylene flasks. Finally, the solution was ready for reading by flame atomic absorption spectrophotometer [7].

$$Econ = A \times B / C$$

Econ: Concentration of metal in water (mg/l).

A: Concentration of metal in calibration curve (mg/l).

B: Final volume of sample (ml).

C: Initial volume of sample (ml).

Examined fish histologically by histological dissection from gills fish, samples were collected from gill fish and fixed in 10% formalin in test tube for 24h, dehydrated in graded ethanol material concentrations and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and putted on glass slides. Sections were deparaffinized in xylene, dehydrated in ethanol material, stained with Hematoxylin and Eosin (HE) stain for examined pathological changes by light microscope [6].

Results

The fishes were collected from river showed behavioral abnormalities such as: increase movement, erratic swimming, low in respiration rate, convulsion, loss of equilibrium,

increase in mucous on skin fish and gills, appeared dark red color and little from congestion on gill surface (gill arch and gill lamella). Some cases appeared on fish specific gross signs such as (yellow or green pustules on gill and muscle) yellow spots on gills, spots that increase in size and develop to brown tissue dies, leaving a hole in the gill structure) these signs indicate the presence of necrosis of the gill fish exposed to pollution (Fig.1). The results in present study showed that the concentration of dissolved Pb in water was very high (0.39) µg/l compared with Cd was reached to (0.05) µg/l, Co (0.01) µg/l and Cu (0.154) µg/l.

Table.1. illustrated the variations of the heavy metal concentration dissolved in water at all months, ranged between the lowest value Co (0.01µg/l), and the highest value Pb (0.39µg/l).

Results explained the values of heavy metals concentration in the order Pb (0.39) µg/l > Cu (0.154) µg/l > Cd (0.05) µg/l > Co (0.01) µg/l.

Results showed that the concentration values of Pb dissolved in water recorded more than Cd in this station.

The results of histopathological dissection on fish exposed to environmental pollution, the gills showed great dilation and congestion of Central Venus Sinus and severe cellular infiltration, (Fig.2) accompanied with severe fusion in secondary lamella, cellular infiltration in secondary lamella and mild congestion of secondary lamella, (Fig.3). In another slide showed shortening of secondary lamella and slight cellular infiltration in some secondary lamella with dilation of C.V.S. (Fig.4). Fish gills exposed to environmental pollution appeared moderate epithelial lifting and cellular infiltration in secondary lamella in (Fig.4) and (Fig.5). The gill section showed severe vacuolation of epithelial secondary lamella and mononuclear cell infiltration in secondary lamella, (Fig.6). Slight cellular infiltration in some secondary lamella and severe depletion of hemopoitic tissues, (Fig.7).

Table 1: Concentrations of heavy metal in Diyala River water and comparison with guidelines and (mean ± SD.) (µg/L).

Guidelines/	Cd	Pb	Co	Cu	Fe	Ni	Reference
Diyala River	0.05	0.39	0.01	0.154	This study
WHO	0.01	0.05	...	2	...	0.02	WHO,1993
EPA	0.01	0.05		1.3	0.3	...	EPA,2002
WPCL	0.003	0.01		0.02	0.3	0.02	WPCL,2004
Al-masab Alamm River	0.02-0.09	0.005-0.6	0.2-0.6	0.006-0.1	109-200.4	0.05-2.6	Al-Awady, 2011
Tigers River	0.02-0.06	0.8-1.2	Al- Samawihis,2017
EC	5	10	...	2	0.2	20	EC,1998
TSE-266	0.005	0.01	...	2	0.2	0.02	TSE-266,2005
Siberian pond	<0.001	0.002	...	0.002	0.29	0.002	Gladyshev <i>et al.</i> ,2001



Fig 1: necrosis of the gill (yellow to brown gill lamella).



Fig 2: Microscopical section of gills fish showed severe dilation and congestion of Central Venus Sinus (↔) and severe cellular infiltration (→) (H&Ex40).

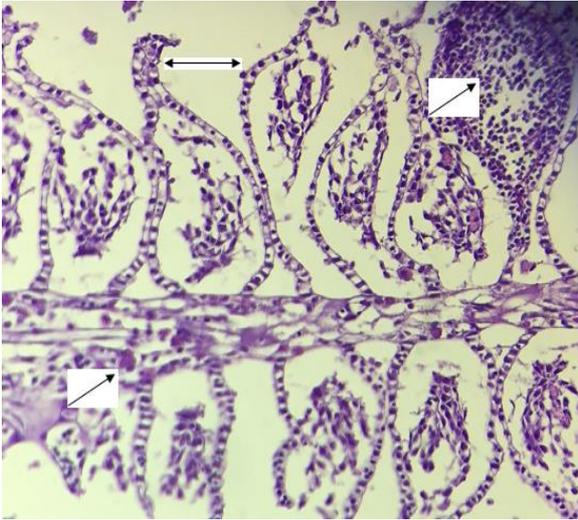


Fig 3: Microscopical section of gills fish showed severe fusion in secondary lamella, (↔) cellular infiltration in secondary lamella and mild congestion of secondary lamella, (→) (H&Ex40).

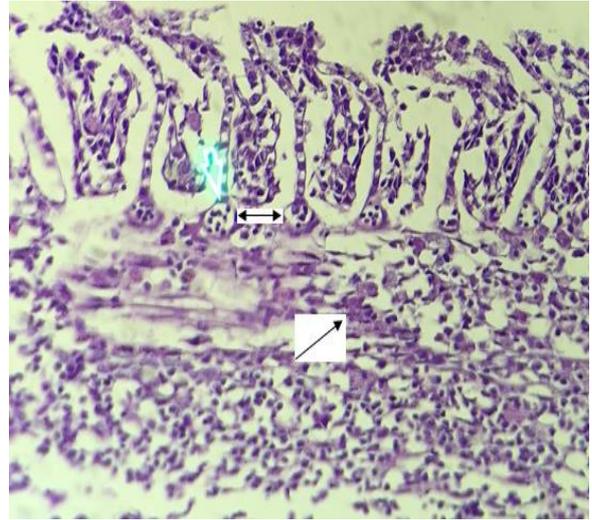


Fig 6: Microscopical section of gills fish showed severe vacuolation of epithelial secondary lamella (↔) and mononuclear cell infiltration (→) (H&Ex40).

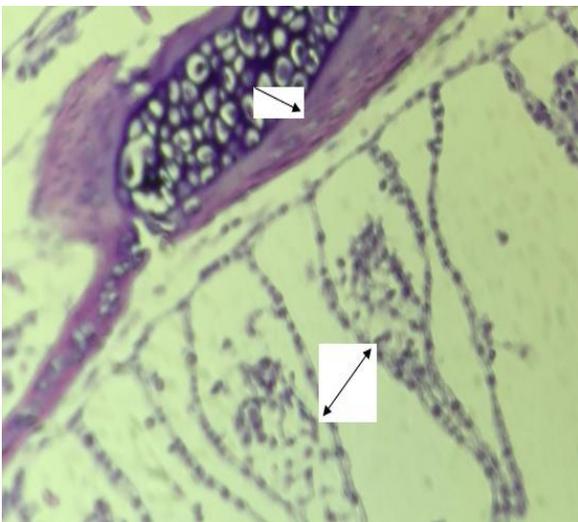


Fig 4: Microscopical section of gills fish showed shortening of secondary lamella and slight cellular infiltration in some secondary lamella (↔) with dilation of C.V.S. (↔) (H&Ex40).

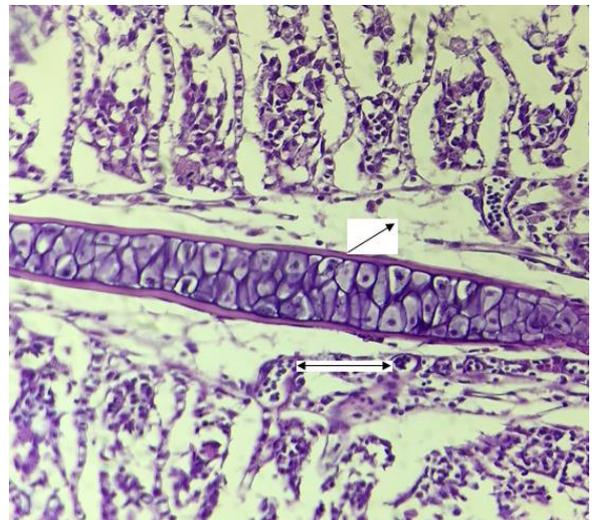


Fig 7: Microscopical section of gills fish showed slight cellular infiltration in some secondary lamella (↔) and severe depletion of hemopoietic tissues, (→) (H&Ex40).

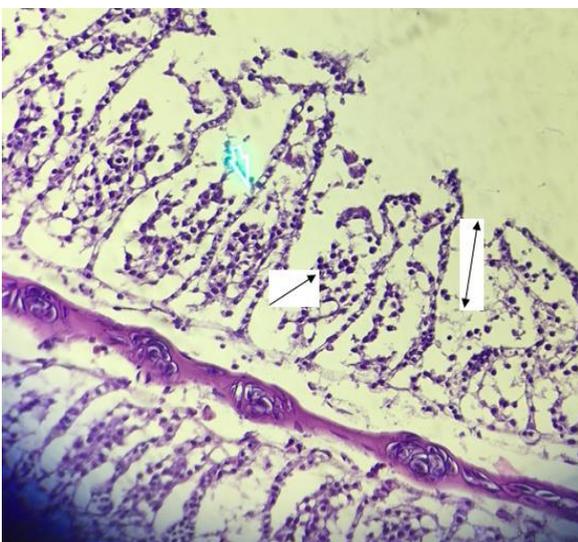


Fig 5: Microscopical section of gills fish showed moderate epithelial lifting (↔) and cellular infiltration in secondary lamella (→) (H&Ex40).

Discussion

Municipal solid waste, industrial effluents, human interference with their resultant negative effects such as changes in water quality may identified to be major reasons for increasing percentage of pollution and appeared many of abnormal clinical signs on fish which grew in this area of river such as increased quantity of mucous on fish gills and skin, appeared dark red color and little from congestion on gill surface.

Appeared increase the mucous on skin surface and gill fish, the mucous membrane considers the first define line in immunological response in all fish and in all animals so that this sign (increased mucous) occurred because stimulate the immune system and this considers the first immunological response against the environmental pollution [16].

The heavy metals characterized by accumulated inside organism bodies [17 18]. The accumulation levels in organism effected by different environmental factors, different seasons years, culturing location, reproduction periods in fish, water temperature, water salinity and water turbidity [19]. Resulting from that accumulate most pollutants in muscle fish lead to great dangerous on fish health and that effect not only on fish

but may reach to human if not found methods to treated fish and removed any pollutant factor may found in environment fish (water quality). Fishing rate characterized by increased in January and February month but decreased in March and April month because found relation between fishing amount and water temperature, this result agreed with ^[20, 21].

Decrease fishing amount in March and April belong to increase water temperature and this agreed with ^[22]. which referred to decrease fishing amount in salmon during summer season belong to elevation in water temperature which decrease from active and movement of fish, and that's effect on number of fishes in fishing grids. Resulted from this study, the present high level of interferences in this area of river including human disturbances such as swimming, bathing washing and slaughter of cattle and sheep which resulted in accumulation sediment and high levels from heavy metals in this station.

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Conclusion

The water pollution and effect on freshwater fish depends on numerous factors, such as environmental concentration of heavy metals, environmental factors (pH, dissolved oxygen hardness and water temperature), exposure duration and feeding habits (carnivorous, herbivorous and omnivorous).

Histological changes are more sensitive and important than any other biochemical parameter. They provide important technique of fish health and to the effect of pollution on each parameter.

The results obtained from this study clearly appeared from necessary control on the used methods of environmental pollution such as pesticide, insecticide, industrial waste, sewage waste and a heavy metal such as cadmium, lead and mercury.

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