Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Comparison between heavy metal residues and their effect on African Catfish *(Clarias gariepinus)* and Nile Tilapia (*Oreochromis niloticus*)

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Received in 16/5/2023
Received in revised from 30/5/2023
Accepted in 21/6/2023

Keywords:
Heavy metal
African catfish
Nile Tilapia

ABSTRACT:

Fish are an important source of proteins of a high value. Bioaccumulation of heavy metals in aquatic animals causes serious threat for fish as well as human via persistent consumption of fish. This study aims to estimate the concentrations of some heavy metals (lead (Pb), cadmium (Cd) and chromium (Cr)) in fish muscles and water. Samples of fish muscles (*Clariasgariepinus* and *Oreochromisniloticus*) and water were collected from different farms in Sharkia province in Egypt from April to September in 2022. The concentrations of heavy metals had the order Pb> Cr > Cd. The highest values of metals were recorded for Pb in the examined fish while the lowest values were recorded for Cd and Cr. There is a variation in the concentrations of heavy metals in water between investigated farms. The highest values of metals were recorded for Pb in the water. a significant decrease in RBCs count, Hb conc., PCV% and WBCs compared with control group with significant increase in MCV, MCH values in farm B, C and D compared to control and the most pronounced significant changes was correlated to farm C. A significant decrease in phagocytic index and percentage were the recorded effect of heavy metals. The impact of heavy metals on biochemical parameters showed significant increase of AST, ALT, urea, creatinine, glucose and total lipids in the blood of examined fish, but total protein and total globulin, beta and gamma globulin revealed a significant decrease. They also displayed pathological changes in all examined organs.

INTRODUCTION:

Fish are a significant source of polyunsaturated omega-3 fatty acids, several vitamins, mineralsand proteins with a high biological value for the human diet. However, eating fish products can also allow dangerous chemicals like heavy metals to enter the body **(Bakhshalizadeh et al. 2022).** Fish is thought to meet about 60% of the world's protein needs and is low in cholesterol and includes all important amino acids **(Kuton et al. 2021).**

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DOI: 10.21608/ejah.2023.305223

On top of the aquatic food web are the majority of fish species. Since fish may acquire heavy metals more quickly and at a higher concentration than lower order species in the food web, it is an animal that is seriously of worry all over the world. But fish also serves as the finest sentinel for exposing the detrimental impacts of heavy metals on them **(Ullah et al. 2021).**

Cadmium, chromium, nickel, arsenic, copper, mercury, lead, and zinc are the most prevalent heavy metal pollutants that severely toxicity in fishes. The mechanism of metal toxicity is the development of oxidative stress. The immune system is suppressed by oxidative stress, which damages tissues and organs, results in development defects and impairs the ability to reproduce **(Garai et al. 2021).**

The evaluation and monitoring of fish health, stress from pollution and disease depend heavily on haematological research. Studying how contaminants affect fish is important as a result. To gauge the effects of environmental pollution exposure, alterations in haematological markers might be used **(Ahmed et al. 2020).**

The possible negative consequences of metal accumulations in fish can also be detected by biochemical markers. Different enzymes' activity are thought to be sensitive biochemical indicators before harmful effects manifest in fish, and they are crucial factors for analysing water for the presence of toxins **(Al-Asgah et al. 2015).**

Population extinction occurred as a result of many factors and known via the pathological picture of the organs **(Fahmi et al. 2019).**

This study aim to determine the concentrations of some heavy metals (Pb, Cd and Cr) in fish muscles and water from different farms.In addition to the impact of metals on hematological and biochemical parameters of fish as well as the pathological changes.

MATERIALS AND METHODS

Ethical approval

The animal study was approved by the committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine**, Zagazig University (ZU-IACUC/2/F/167).**

Study area:

Fish samples (African Catfish and Nile Tilapia) and water samples were collected from four different farms at Sharkia governorate farm (A) at Kafr El-Hosr Bridge, farm (B) at Muweis canal, farm (C) at veterinary Zagazig University and farm (D) at Abo Hammad.

Sample collection:

100 African Catfish and Nile Tilapia were caught bimonthly by gill net from the selected sites (50 fish from each species). Body weight ranging from 250-300 g for African catfish and 150-200g for Nile tilapia. 20 water samples were collected from selected farms (5 samples from each). Twenty African Catfish and Nile Tilapia were collected and transferred in tanks for acclimatization for one month at a glass aquarium each including 10 fish and kept as control groups. Fish specimens were free from any parasites after parasitological examination at Department of Parasitology, Faculty of Veterinary Medicine Zagazig University. Muscles sample and water samples were frozen and stored at -20 until assayed for Heavy metal residues.

Heavy metal residues:

The analysis of tissue sample was represented by one gram of each sample was macerated by sharp scalpel in a screw capped tube 5 ml of acid digestion mixture (3 ml HNO_3: $2HCIO_4$) were added to the tissue sample **(Zantopoulos et al. 1996)**. Tubes used were tightly closed and the content was vigorously shaken and allowed to stand overnight at room temperature. Then, these tubes were heated for 3 hours in water bath adjusted at 70^{o}C to ensure complete digestion of samples. The digestion tubes were vigorously shaken at 30 minutes intervals during the heating period. Finally, the tubes were cooled at room temperature and then each tube was diluted with 20 ml de-ionized water and filtered by using filter paper (Watt man No.42). The filtrate was collected in glass tubes and these tubes were capped with polyethylene films and kept at room temperature until analysis for heavy metal contents

(Tsoumbaris, 1990). Heavy metal concentrations in water were determined by atomic absorption spectrophotometer according to **Mansour et al. (2019).** Analysis of lead, cadmium and chromium was conducted at Central Laboratory, Faculty of Veterinary medicine, Zagazig University. It was conducted by air / Acetylene flow (5.5/1.11/m) flame (A.A.S).

Blood samples:

Blood samples (50) were collected from caudal vein under aseptic precautions from fish. The first sample was 1 ml of blood collected on EDTA to determined red blood cells (RBCs), hemoglobin (Hb) concentration and total leukocytic counts were determined according to the routine hematological procedures as described by **Feldman et al. (2000).** Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to (Dacie and Lewis, 2002). The second blood collected on heparin (50 IU/ml) for Phagocytic activity and phagocytic index described by Goddeeris et al. (1986). The third blood sample was taken without anticoagulant for biochemical analysis.

Biochemical studies:

All biochemical parameters were carried out using commercial kits, the used protocol for each parameter was done as recommended by the manufacture manual. The liver transferases (alanine aminotransferase ALT and aspartate aminotransferase AST) activities were estimated according to **Kaplan and Pesce (1984).** Serum urea was determined according to **Kaplan and Pesce (1984)** and the serum creatinine was estimated according to Henry (1964). Total cholesterol level was carried out according to **White et al. (1970).** Serum glucose was measured according to **Trindax (1969).** The serum total protein was tested according to **Tietz (1995).** Electrophoretic analysis was carried out for determination of serum albumin, alpha, beta and gamma globulins according to the technique described by **Davis (1964).**

Pathological examination:

The macroscopic findings as well as the microscopic findings were recorded. Tissue specimens from liver, kidney, intestine , gills and skeletal muscles from African Catfish and Nile Tilapia were fixed in neutral buffered formalin 10% for 48 hours, dehydrated in ascending grades of ethanol (70%-100%), cleared in xylene, embedded in paraffin wax. 5µm thickness of paraffin sections were obtained by using automated microtome then stained with routine Hematoxylin and Eosin (H & E) **(Suvarna et al. 2018).**

Statistical analysis:

The analysis of variance was used in the statistical analysis (ANOVA). At a significant threshold of 0.05, Duncan's Multiple Range was employed to identify changes in the treatment groups. The SPSS application was used on a PC to run all statistics **(SPSS, 2004).**

RESULTS:

Heavy metals in fish muscles

Comparing the mean concentrations of some heavy metals in fish muscles of African Catfish and Nile Tilapiain different farms are shown in table **(1&2)**. The concentrations of heavy metals had the order Pb> Cr > Cd. The highest values of metals were recorded for Pb in the examined fish as in farm C while the lowest values were recorded for Cd and Cr. In addition to the concentrations of heavy metals in the control groups were below the detection limit.

Heavy metals in water

Comparing the concentrations of some heavy metals in water in different farms are shown in table **(3)**. There are variations in the concentrations of heavy metals between investigated farms. The highest values of metals were recorded for Pb in the water.

Table 1. Concentration of some heavy metals in the muscles of African Catfish harvested from different farms (mean ± SE).

Farms / Heavy metals	A	B	C	D
Pb (µg/g)	0.016±0.005[d]	0.600±0.050[b]	1.670±0.040[a]	0.256±0.030[c]
Cd (µg/g)	0.008±0.003[b]	0.024±0.006[ab]	0.008±0.005[b]	0.049±0.010[a]
Cr (µg/g)	0.030±0.005[b]	0.042±0.005[b]	0.174±0.030[a]	0.138±0.050[a]

*Notes: Permissible limit concentrations by WHO = 0.01 µg/g (Pb) , 0.01 µg/g (Cd) and 0.0 (Cr) µg/g. Different letters at the same row means that there was a significant change at $p<0.05$, n=5.

Table 2. Concentration of some heavy metals in the muscles of Nile Tilapia harvested from different farms (mean ± SE).

Farms / Heavy metals	A	B	C	D
Pb (µg/g)	0.012±0.005[b]	0.268±0.12[a]	0.296±0.090[a]	0.100±0.05[ab]
Cd (µg/g)	0.000 (BDL)	0.000 (BDL)	0.018±0.003[a]	0.012±0.005[a]
Cr (µg/g)	0.022±0.003	0.016±0.009	0.024±0.020	0.012±0.005

*Notes: Permissible limit concentrations by WHO = 0.01 µg/g (Pb) , 0.01 µg/g (Cd) and 0.0 (Cr) µg/g. Different letters at the same row means that there was a significant change at $p<0.05$, n=5
*BDL=below the detection limit

Table 3. Concentration of some heavy metals in water samples collected from different farms (mean ± SE).

Farms / Heavy metals	A	B	C	D
Pb (µg/L)	0.018±0.0300[b]	0.001±0.0002[b]	0.188±0.0300[a]	0.009±0.0006[b]
Cd (µg/L)	0.001±0.0005	0.0001±0.0001	0.003±0.0002	0.0006±0. 0006
Cr (µg/L)	0.000 (BDL)	0.0007±0.0008	0.001±0.0005	0.0003±0.0001

*Notes: Permissible limit concentrations by WHO = 0.1 µg/L (Pb) , 0.001 µg/L (Cd) and 0.1 (Cr) µg/L. Different letters at the same row means that there was a significant change at $p<0.05$, n=5 *BDL = below the detection limit.

Hematological study:

Results of hematological parameters of African Catfish and Nile Tilapia collected from different farms indicated a significant decrease in RBCs count, Hb conc. , PCV% and WBCs compared with control one with significant increase in MCV, MCH values in farm B, C and D compared to control and the most pronounced significant changes was correlated to farm C Table **(4).**

Immunological results:

Data concerning to African Catfish and Nile Tilapia showed significant decrease inphagocytic percentage and index in all farms comparing with control one Table **(4).**

Table 4. Erythrogram, leukogram and phagocyticactivity of African Catfish and Nile Tilapia collected from different farms (mean ± SE).

		African Catfish			
parameters	**control**	**A**	**B**	**C**	**D**
RBCs(10^6×mm3)	2.22±0.20^{a}	2.09±0.17^{b}	2.04±0.22^{b}	1.49±0.12^{c}	1.88±0.15^{bc}
Hb(gm/dl)	11.66±0.33^{a}	11.20±0.18^{b}	11.26±0.20^{b}	8.65±0.30^{d}	10.59±0.28^{c}
PCV%	23.02±0.36^{a}	22.08±0.25^{b}	22.18±0.46^{b}	21.28±0.28^{c}	21.51±0.33^{c}
MCV	103.70±1.40^{c}	105.64±2.19^{c}	108.73±2.50^{b}	142.82±1.75^{a}	114.41±2.85^{b}
MCH	52.53±1.09^{b}	53.59±1.14^{b}	55.20±0.95^{ab}	58.10±1.02^{a}	56.33±0.93^{a}
MCHC	50.66±1.16^{a}	50.72±0.75^{a}	50.77±1.32^{a}	40.65±0.98^{b}	49.25±0.90^{a}
WBCs(10^3×mm3)	20.16±1.70^{a}	17.20±1.15^{b}	16.52±.90^{b}	12.70±0.79^{d}	14.95±0.75^{c}
Phagocytic %	74.12±1.50^{a}	67.00±1.04^{b}	66.75±1.50^{b}	56.85±1.70^{c}	64.43±1.95^{b}
Phagocytic index	3.60±0.23^{a}	3.15±0.20^{b}	2.90±0.18^{c}	1.88±0.15^{d}	2.80±0.26^{c}
		Nile Tilapia			
parameters	**control**	**A**	**B**	**C**	**D**
RBCs(10^6×mm3)	6.76±0.12^{a}	4.10±0.26^{c}	5.76±0.16^{b}	3.02±0.16^{d}	3.95±0.31^{c}
Hb(gm/dl)	18.61±0.56^{a}	12.17±0.72^{b}	11.87±0.13^{b}	9.38±0.24^{c}	11.88±0.82^{b}
PCV%	48.35±0.77^{a}	34.72±1.37^{b}	34.54±0.26^{b}	27.62±0.79^{c}	34.50±1.22^{b}
MCV	71.52±1.27^{c}	84.68±2.32^{b}	59.97±1.25^{d}	91.46±2.37^{a}	87.34±2.18^{b}
MCH	27.53±0.44^{b}	29.68±0.27^{a}	20.61±0.37^{c}	31.06±0.88^{a}	30.08±0.25^{a}
MCHC	38.49±0.72^{a}	35.05±0.78^{b}	34.37±0.18^{b}	33.96±0.15^{b}	34.43±1.22^{b}
WBCs(10^3×mm3)	14.89±0.19^{a}	9.50±0.58^{c}	12.42±0.25^{b}	7.79±0.18^{d}	9.50±0.58^{c}
Phagocytic %	81.60±2.91^{a}	77.60±3.00^{b}	76.40±2.44^{b}	70.30±1.73^{d}	74.00±1.89^{c}
Phagocytic index	2.63±0.13^{a}	2.36±0.16^{b}	2.27±0.13^{bc}	1.90±0.11^{d}	2.16±0.16^{c}

Different letters at the same row means that there was a significant change at $p<0.05$, n=5.

Biochemical results:

Biochemical parameters of African Catfish and Nile Tilapia collected from different farms are illustrated in table **(5).** Serum activity of AST, ALT, urea, creatinine, glucose and total lipids showed the highest value in farms C and D in the serum of African catfish compared with control one. AST and ALT showed significant increase in blood serum of Nile tilapia collected from farms C and B while serum urea and creatinine revealed significant increase in all farms in compared with control one. Also serum glucose and total lipids reflected re-

markable increase in serum of Nile Tilapia collected from farms C and B.

Protein electrophoresis results:

The results of Protein electrophoresis of African Catfish presented in table **(5)** revealed a significant decrease in total protein and total globulin, beta and gamma globulin in all farms compared to control but a significant reduction in serum albumin in farm C compared to control group and alpha globulin showed significant decrease in farm C and D compared with control.

The results of Protein electrophoresis of NileTilapia showed a significant decrease in total protein, albumin and total globulin as well as alpha, beta and gamma globulin in all farms compared to control one table **(5)**.

Table 5. Some biochemical parameters and protein profile of African Catfish and Nile Tilapia collected from different farms (mean ± SE).

		African Catfish			
parameters	control	A	B	C	D
AST (IU/L)	14.08±1.08^{c}	26.11±2.9^{c}	24.11±0.94^{c}	97.41±3.2^{a}	74.67±8.1^{b}
ALT (IU/L)	18.99±0.5^{d}	37.93±4.1^{c}	43.13±2.6^{c}	149.72±6.6^{a}	128.66±1.8^{b}
Urea (mg/dl)	2.53±0.08^{d}	3.53±0.08^{b}	2.94±0.04^{c}	4.10±0.1^{a}	3.70±0.05^{b}
Creatinine(mg/dl)	0.47±0.04^{d}	0.81±0.05^{c}	0.59±0.06^{d}	1.26±0.04^{a}	1.03±0.08^{b}
Glucose(mg/dl)	46.15±0.3^{e}	60.23±2.70^{d}	67.33±1.40^{c}	95.50±2.70^{a}	74.17±1.70^{b}
T.lipid (mg/dl)	127.38±2.7^{d}	229.38±0.85^{c}	238.13±5.02^{c}	337.90±6.10^{a}	286.20±8.90^{b}
Total protein(g/dL)	4.48±0.12^{a}	4.00±1.02^{b}	4.15±0.82^{ab}	3.39±0.95^{d}	3.67±0.52^{c}
Albumin (g/dL)	2.15±0.09^{a}	2.15±0.08^{a}	2.18±0.11^{a}	1.85±0.08^{c}	1.95±0.06^{b}
T.globulin (g/dL)	2.33±0.08^{a}	1.86±0.06^{b}	1.97±0.09^{b}	1.54±0.09^{d}	1.72±0.07^{c}
α globulin(g/dl)	0.35±0.03^{a}	0.34±0.02^{a}	0.30±0.02^{ab}	0.22±0.02^{c}	0.27±0.01^{b}
β globulin(g/dl)	0.56±0.02^{a}	0.46±0.01^{b}	0.45±0.02^{b}	0.39±0.01^{d}	0.45±0.03^{b}
γ globulin(g/dl)	1.40±0.04^{a}	1.06±0.03^{c}	1.22±0.04^{b}	0.93±0.03^{d}	1.00±0.03^{c}
		Nile Tilapia			
parameters	control	A	B	C	D
AST (IU/L)	30.16±0.7^{d}	43.91±0.8^{c}	73.50±4.5^{b}	94.25±6.34^{a}	54.83±3.08^{c}
ALT (IU/L)	44.63±2.1^{c}	62.83±0.8^{b}	109.16±6.5^{a}	110.76±5.12^{a}	68.73±3.2^{b}
Urea (mg/dl)	1.66±0.08^{b}	3.05±0.13^{a}	3.48±0.13^{a}	3.50±0.30^{a}	3.21±0.09^{a}
Creatinine (mg/dl)	0.32±0.02^{c}	0.53±0.03^{b}	0.63±0.03^{b}	0.79±0.01^{a}	0.64±0.04^{ab}
Glucose(mg/dl)	49.16±0.40^{c}	56.76±2.30^{b}	67.20±1.40^{a}	72.33±2.40^{a}	59.72±1.40^{b}
T.lipid (mg/dl)	87.48±11.60^{d}	147.06±1.60^{c}	183.60±5.06^{b}	221.06±4.80^{a}	149.83±1.90^{c}
Total protein(g/dL)	5.47±0.22^{a}	4.21±0.40^{b}	4.08±0.52^{b}	3.55±0.31^{c}	3.70±0.28^{c}
Albumin (g/dL)	2.45±0.13^{a}	1.97±0.23^{b}	1.89±0.18^{b}	1.75±0.45^{c}	1.60±0.20^{c}
T.globulin (g/dL)	3.02±0.15^{a}	2.24±0.32^{b}	2.19±0.27^{b}	1.80±0.60^{c}	2.10±0.53^{b}
α globulin(g/dl)	0.65±0.03^{a}	0.33±0.02^{b}	0.32±0.02^{b}	0.30±0.02^{b}	0.35±0.01^{b}
β globulin(g/dl)	0.78±0.02^{a}	0.65±0.01^{b}	0.58±0.03^{c}	0.41±0.02^{d}	0.50±0.02^{cd}
γ globulin(g/dl)	1.59±0.08^{a}	1.26±0.03^{b}	1.29±0.08^{b}	1.09±0.04^{c}	1.25±0.05^{b}

Different letters at the same row means that there was a significant change at $p<0.05$, n=5

Pathological findings in Catfish *(Clarias gariepinus):*

Macroscopic examination revealed variation in gross lesions in different farms according to heavy metals concentration in water and muscles, the gross examination revealed pale color of liver and kidney in addition to reddish color of gills and skeletal muscles,while microscopic examination revealed different pathological changes with variation in severity among difrent farms, the score lesions of catfish collected in difrent farms presented in table **(6)**.

Microscopic examination of sections from hepatopancreas of catfish showed degeneration and necrosis in groups of hepatocytes beside necrotic areas of pancreatic acini with pyknotic nuclei **(fig.1A).** Other sections of hepatopancreas revealed intense fatty degenerations in most hepatocytes in addition to congested hepatic vasculatures **(fig.1B).**

Necrotic some renal tubules and hypocellular glomerular structures were commonly observed lesions beside presence of interstitial hematopoietic cells in kidney **(fig.2A)**. In addition, dilated renal blood vessels were also seen **(fig.2B)**.

Sections from Intestine showed denuded lining epithelium with exudation within intestinal lumina, beside dilated submucosal blood vessels **(fig.3A)**. Other sections demonstrated ulcerated mucosal surface with heavy inflammatory infiltrates and accompanied with dilated submucosal and serosal vasculatures **(fig.3B).**

Sections from gills revealed masses of detached primary lamellae admixed with inflammatory exudates within interstitial tissue between gill lamellae beside adhered some secondary filaments with each other in adjacent primary lamellae **(fig.4A)**. Other sections of gills showed congested gill capillaries within primary lamellae **(fig.4B)**.

Sections from skeletal muscles revealed vacuolated myocytes **(fig.5A)**. Interstitial hemorrhages between destructed muscles were also observed **(fig.5B).**

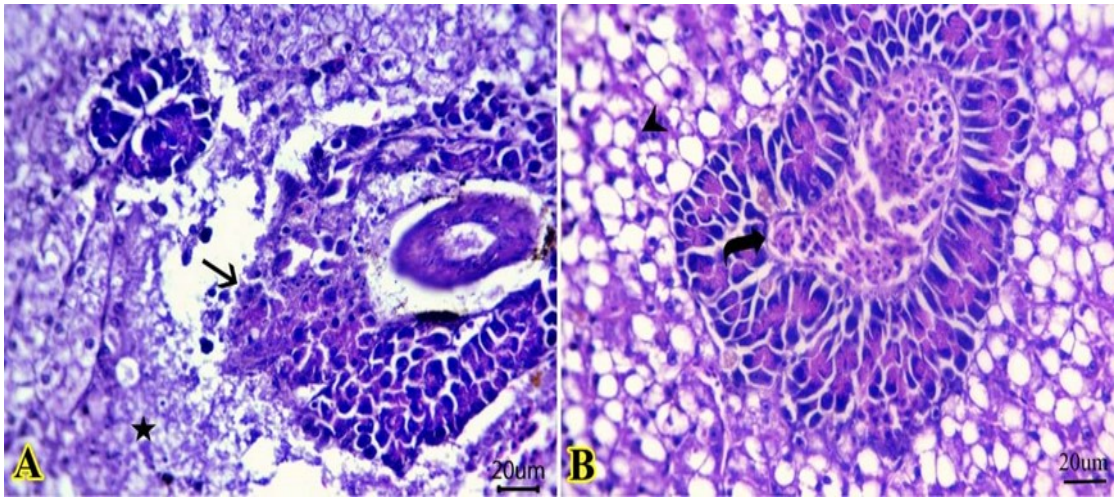

Fig.1: Photomicrograph of H&E stained sections from hepatopancreas **(Scale bar 20µm)** showing: **A:** degeneration and necrosis in groups of hepatocytes **(star)** beside necrotic areas of pancreatic acini with pyknotic nuclei **(arrow). B:** intense fatty degenerations in most hepatocytes **(arrowhead)** in addition to congested hepatic vasculature **(curved arrow)**.

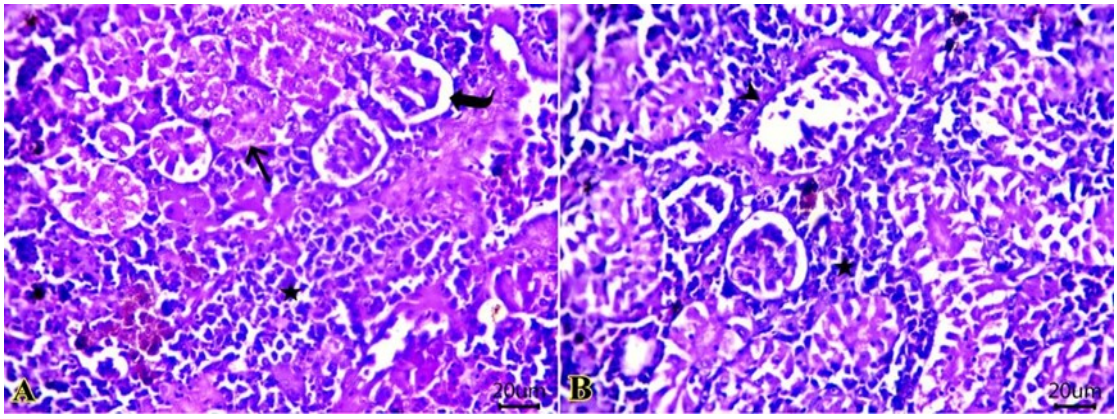

Fig.2: Photomicrograph of H&E stained sections from kidney **(Scale bar 20µm)** showing: **A:** Necrotic some renal tubules **(arrow)** and hypocellularglomerular structures **(curved arrow)** beside presence of interstitial hematopoietic cells **(star)** and melanomacrophages**. B:** dilated renal blood vessels **(arrowhead)**.

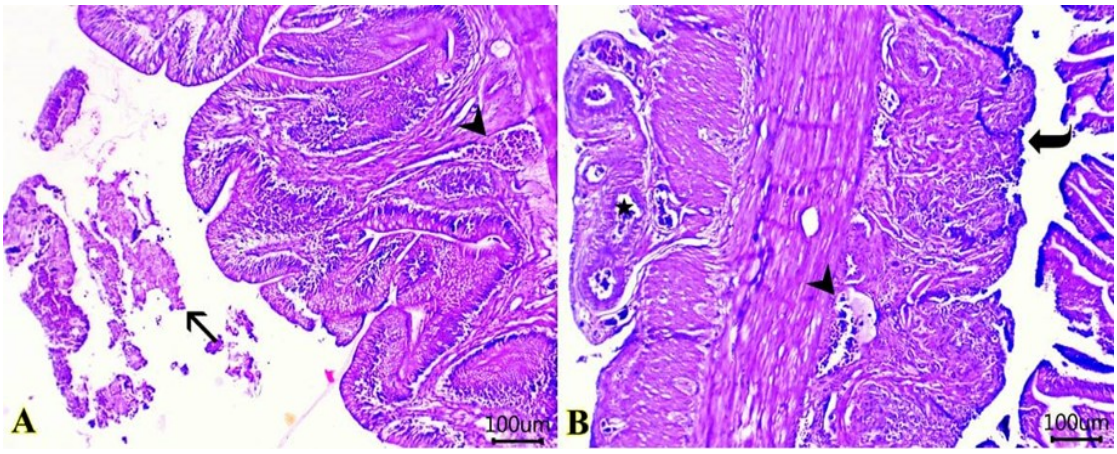

Fig.3: Photomicrograph of H&E stained sections from intestine **(Scale bar 100µm)** showing: **A:** denuded lining epithelium with exudation within intestinal lumina (arrow), beside dilated submucosal blood vessels **(arrowhead). B:** ulcerated mucosal surface with heavy inflammatory cells infiltrates **(curved arrow)** and dilated submucosal **(arrowhead)** and serosal blood vessel **(star).**

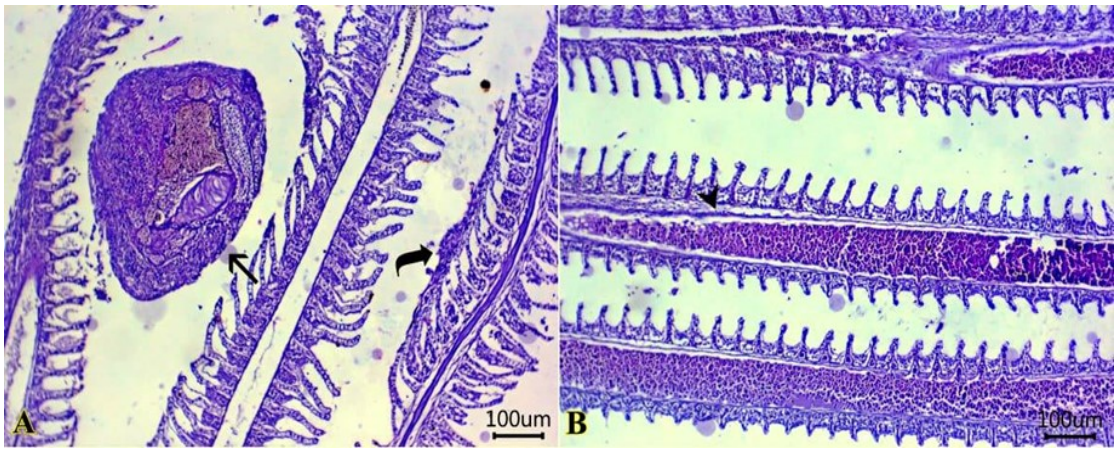

Fig.4: Photomicrograph of H&E stained sections from gills. **(Scale bar 100µm)** showing: **A**: mass of detached primary lamellae admixed with inflammatory exudates within interstitial tissue between gill lamellae **(arrow)** beside adhered some secondary filaments with each other in adjacent primary lamellae **(curved arrow). B:** congested gill capillaries within primary lamellae **(arrowhead)**.

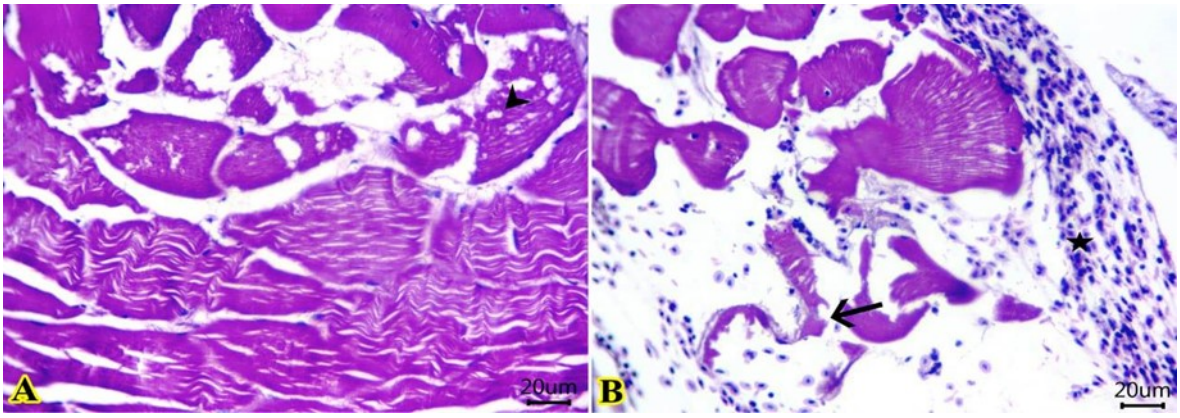

Fig.5: Photomicrograph of H&E stained sections from skeletal muscles **(Scale bar 20µm) A:**showing vacuolated myocytes **(arrowhead**). **B:** destructed muscles **(arrow)** and interstitial hemorrhages **(star).**

Pathological findings in Nile Tilapia (*Oreochromis niloticus*)

Macroscopic examination revealed variation in gross lesions in different farms according to heavy metals concentration in water and muscles, the gross examination revealed yellowish colour of liver while kidney revealed pale colour in some areas and dark colour in other areas in addition to dark colour of gills and reddish colour of muscles, while microscopic examination revealed different pathological changes with variation in severity among different farms, the score lesions of Nile Tilapia collected in difrent farms presented in table **(7)**.

Microscopic examination of hepatopancreas (**fig.6A)** showed dilated hepatic sinusoids and other vasculatures. Other examined sections **(fig.6B)** showed fatty change within hepatic and pancreatic acini beside presence of areas of inflammatory edema.

Kidney **(fig.7A)** revealed necrotic renal tubular epithelium in large number of tubules which devoid cytoplasm with pyknotic nuclei, cellular depletion within some glomerular tufts and thickened wall of renal blood vessel. Further, dilated renal blood vessels were seen in some examined tissue **(fig.7B)**.

Intestine **(fig.8A)** exhibited desquamated sheets of enterocytes. As well, edematous submucosal layers were detected in some sections **(fig.8B).**

Gills **(fig.9A)** showed exudates and detached secondary lamellae in between primary gill filaments. Other sections **(fig.9B)** revealed dilated gill capillaries within some primary lamellae.

Skeletal muscles showed degenerated and destructed myocytes**(fig.10A).** In addition, interstitial hemorrhages were also seen in some examined sections **(fig.10B).**

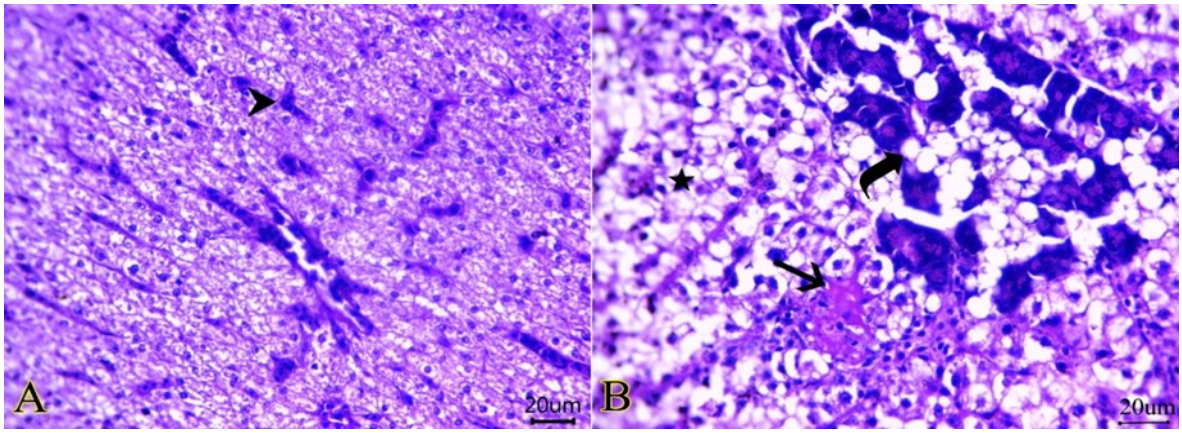

Fig.6: Photomicrograph of H&E stained sections from hepatopancreas. **(Scale bar 20µm)** showing : **A**: dilated hepatic sinusoids **(arrowhead)** and other vasculatures. **B**: fatty change within hepatic **(star)** and pancreatic acini **(curved arrow)** beside presence of areas of inflammatory edema **(arrow).**

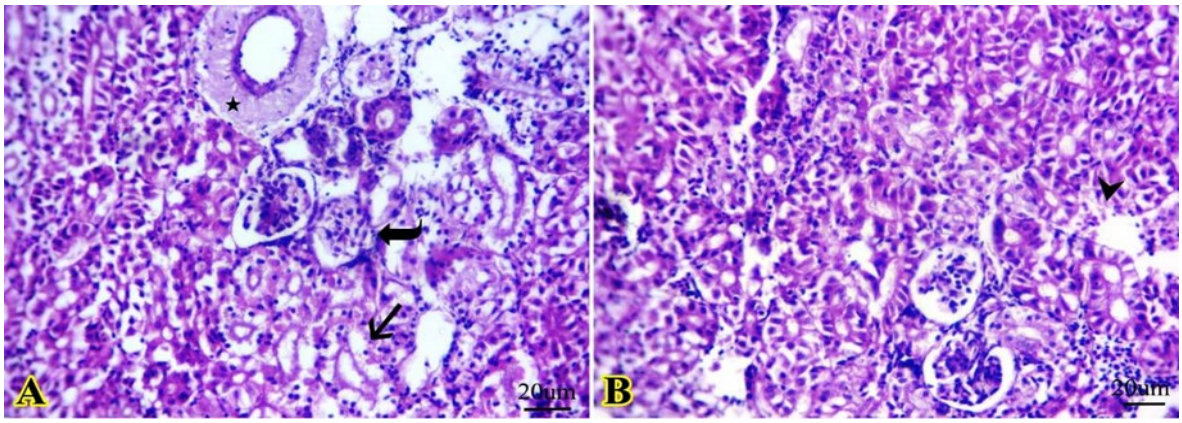

Fig.7: Photomicrograph of H&E stained sections from Kidney **(Scale bar 20µm)** showing : **A**: necrotic renal tubular epithelium with devoid cytoplasm and pyknotic nuclei **(arrow)** , cellular depletion within some glomerular tufts **(curved arrow)** and thickened wall of renal blood vessel **(star). B**: dilated renal blood vessel **(arrowhead)**.

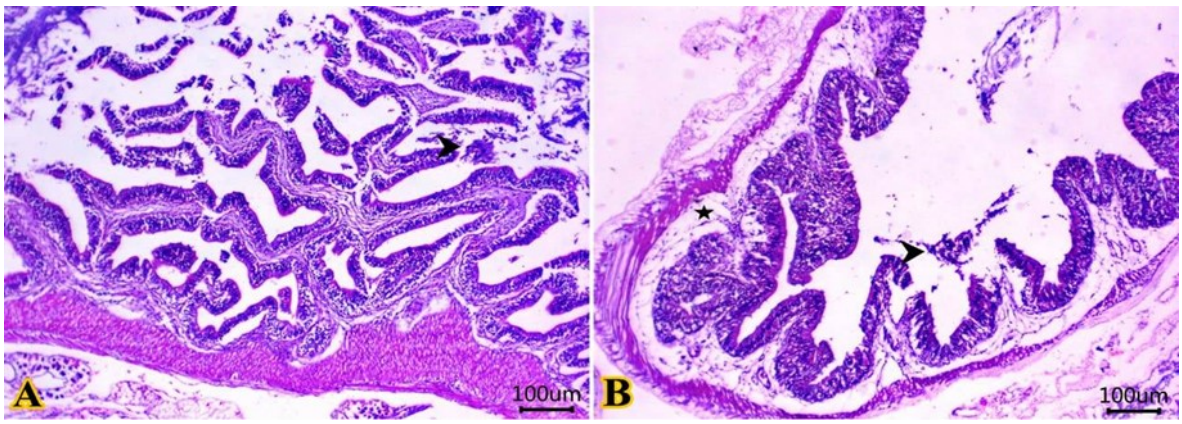

Fig.8: Photomicrograph of H&E stained sections from Intestine **(Scale bar 100µm)** showing: **A:** desquamated sheets of enterocytes **(arrowhead). B:** edematous submucosal layers **(star)**.

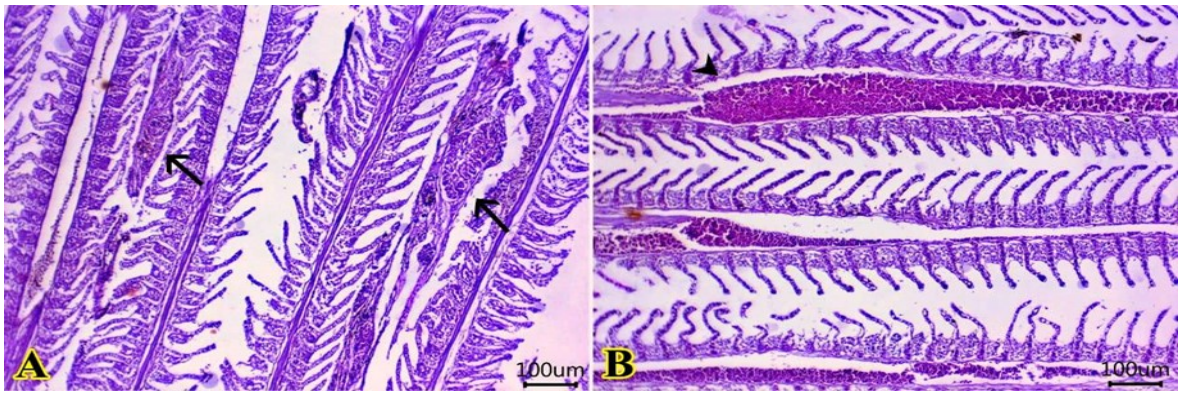

Fig.9: Photomicrograph of H&E stained sections from gills **(Scale bar 100µm)** showing: **A:** exudates and detached secondary lamellae in between primary gill filaments **(arrows). B**: dilated gill capillaries within some primary lamellae **(arrowhead)**.

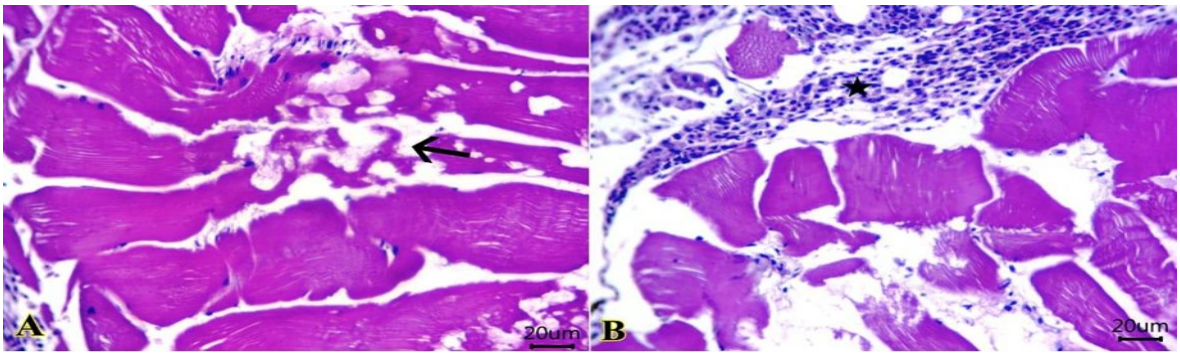

Fig.10: Photomicrograph of H&E stained sections from skeletal muscles **(Scale bar 20µm) A**:showing degenerated and destructed myocytes **(arrow)**. **B:** interstitial hemorrhages **(star)**.

Table 6. Lesions score of the severity extent in liver, kidney, intestine gills and muscles tissues among Catfish farms

Organ	Lesions	A	B	C	D
liver	Vacuolated hepatocytes	++	++	+++	++
	Fatty changes	+	++	+++	++
	Necrotic hepatic cells	+	+	++	+
	Necrotic pancreatic cells	+	+	+	+
	Dilated vasculatures	++	+	++	+
kidney	Degenerated& necrotic renal tubules	+	++	+++	++
	Shrinked glomeruli	+	+	++	+
	Lymphocytic infiltrates	++	++	+++	++
	Dilated blood vessels	++	+	++	+
intestine	Desquamated epithelium & exudates	+	++	+++	+
	Ulcerated mucosa	+	+	++	+
	Dilated blood vessels	+	++	++	++
	Inflammatory cells infiltrates	+	+	+	+
Gills	Detached primary lamellae	++	++	+++	++
	Inflammatory exudates	+	+	++	+
	Gill filament adhesion	+	++	++	+
	Congested gill capillaries	+	++	+++	++
Skeletal muscles	Degenerated myocytes	+	+	+++	+
	Necrotic myocytes	-	+	++	+
	Interstitial hemorrhages	+	+	++	++

Notes: (-) no lesions, (+) mild lesion, (++) moderate lesions, (+++) severe lesions

Table 7. Lesions score of the severity extent in liver, kidney, intestine gills and muscles tissues among Tilapia farms:

Organ	Lesions	A	B	C	D
liver	Hepatocytic&pancreatic fatty change	+	++	+++	++
	Areas of inflammatory edema	++	+	++	+
	Necrotic changes	+	+	+	++
	Dilated sinusoids &other blood vessels	+	+	++	+
kidney	Necrotic renal tubules	+	+	++	+
	depleted glomerular tufts	++	++	+++	+
	Thickened wall of renal blood vessels	+	+	+++	+
	Dilated renal blood vessels	++	+	++	+
intestine	Desquamated sheets of enterocytes	++	++	+++	++
	Edematous submucosa	+	+	+++	++
	Inflammatory cells infiltrates in intestinal wall	+	++	+	+
Gills	Detached secondary lamellae	+	+	++	++
	Exudates between primary lamellae	+	++	+++	+
	dilated gill capillaries	++	+	+++	++
Skeletal muscles	Degenerated musculatures	++	++	+++	++
	Necrotic musculatures	-	+	++	+
	Interstitial hemorrhages	+	++	++	++

Notes:
(-) no lesions, (+) mild lesion, (++) moderate lesions, (+++) severe lesions

DISCUSSION:

Among animal species, fish are a suitable bioindicators of metal pollution due to their position at the top of the food chain in aquatic ecosystems **(Solgi and Galangashi, 2018).** The presence of heavy metals in fish muscle is an alarming problem worldwide since fish occupy high trophic food levels and are a significant food source **(Sommer et al. 2002).** In the present study the levels of some heavy metals (Pb, Cd, Cr) identified in fish muscles and water at different farms. Metals levels are correlated with ambient metals concentration in the surrounding environment, the available metal form in water, the structure of the target organ as well as the interaction between the metal and this organ **(Gupta and Singh, 2011).** The concentrations of heavy metals in muscles had the order Pb> Cr >Cd similarresults obtained by **El-Sayed et al. (2011)** who found that the variations between metals may be attributed to the amount and source of pollution from an area to another.

The bioaccumulation of Pb was higher in both fish species than the other examined metals but the highest value recorded in Clariasgariepinus muscles (1.67±0.04 µg/g) in farm C. Also the concentration of Cr and Cd were higher in Clariasgariepinus than in Oreochromisniloticus. These results agree with **Mansour et al. (2019)** who reported that this may be due to the difference in the feeding habits of the two species. Where O. niloticusprimarily feeds on phytoplankton.

Pb is a serious environmental contaminant and is toxic to fish and human even in small quantities **(Rajeshkumar and Li, 2018).** The lower concentration of Cd in muscle observed in this study suggests that muscle tissue is not an active site for the Cd accumulation process **(Mahjoub et al. 2021).**

The highest levels of heavy metals were detected in water samples collected is Pb The water content of heavy metal may be attributed to wastes of industrial activities and the nature of water properties changed by pollution **(El-Sayed et al. 2011).** Chromium levels were relatively high in tissues as compared to the concentration found in the water same result obtained by **Oldewage and Marx** (2000) and that could be suggestive of a large quantity of

Cr uptake via the food chain because of the omnivorous and bottom feeding habits of C. gariepinus.

Previous researches pose disruptive effects of heavy metals including lead, cadmium, chromium, etc. on different biological systems of fish species such as hormonal, physiological, biochemical, histopathological, and hematological systems (**Atli and Canli, 2011; Öner et al. 2008)**. A reduction in the count of red blood cells, hemoglobin, and hematocrit in our study might be attributed to inhibited hemosynthesis or erythropoiesis **(Musa et al. 2013).**

Moreover, **Desai and Parikh (2012)** stated that the decrease in red blood cells might also be associated with their destruction in hematopoietic tissues (demonstrating anemia). Our result agree with **Ullah et al. (2021)** who said that the decrease in the hematocrit and hemoglobin indicated the failed hematopoietic system and osmoregulatory dysfunction under heavy metals stress downstream. The observed disturbance to the hematological profile is congruent to previous studies on heavy metals mediated hematotoxic effects **(Hassan et al. 2018; Massar et al. 2012).** MCH and MCHC values increased in blood of Nile tilapia and African catfish. Similarly, Adeyemo (2007) found significant increase in MCH and MCHC values of African catfish exposed to lead, Also **Osman et al. (2018)** said that MCH and MCHC values increased in blood of Nile tilapia and African catfish collected from the estuaries of the river Nile at Damietta and Rosetta sites (contaminated sites) compared to upstream sites, attributed to a defense reaction against toxicity.

Leukocytes count in blood collected from both African Catfish and Nile Tilapia were significantly decreased. These finding agree with **Jerônimo et al. (2009)** who observed a reduction in the WBC count in fishes collected from polluted river. It was evident previously by **Osman and Kloas (2010)** and **Osman et al. (2012)** that the liver of Nile tilapia and African catfish was the site of maximum accumulation for the heavy metals examined.

Our Study showed a significant reduction of phagocytic percent and phagocytic index in examined fish blood compared with control group. The imbalance antioxidant system and developed oxidative stress produced injury to immune cells by free radical which results in compromised the immune function might be the cause of this reduction. This results explained by **Ghiasi et al. (2010)** who observed a decrease in phagocytic activity in fish and revealed that it may be due to stress reaction of Cd supplementation which lead to increase cortisol level that secreted during stress.

Biochemical results revealed increased levels of AST, ALT, urea, creatinine, glucose and total lipids in the blood of examined fish. These results agree with **Ahmed et al. (2020)** and **El-Khayat et al. (2018)** found an increase in ALT and AST in fish and snail collected from Lake Burullus. Alterations in liver enzymes activities in the serum directly indicates major pathologic changes or liver damage **(Bhattacharya et al. 2008).** The increased levels of AST and ALT in blood plasma indicate impairment of the liver **(Al-Asgah et al. 2015).**

Higher plasma concentrations of creatinine and uric acid can serve as rough indications of renal and glomerular filtration rate, respectively **(Abu et al. 2009).** The increase of urea and uric acid may be due to sewage **(Hassaan, 2011).** Urea is excreted through gills and not via kidneys in fish, therefore the increased urea demonstrated gills dysfunction **(Hassaan, 2011).**

Blood glucose level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions **(Kamal and Omar, 2011).** Heavy metals causing hyperglycemia by activating the glycogenolysis in fish **(Levesque et al. 2002).** Glucose has been investigated as a sensitive and an indicator of pollutants producing environmental stress in fish brought on by physical factors **(Manush et al. 2005**). Changes in glucose metabolism were caused by stress factors such hunger, hypoxia, and heavy metal toxicity **(Tuncsoy et al. 2014).**

Ullah et al. (2021) stated that a higher

amount of cholesterol was released into the blood as a result of kidney or liver failure, which may be the cause of the cholesterol increase. It could also be linked to the permeability of the hepatic cells, as in cases where the bioaccumulation of heavy metals in the liver caused disturbances in lipid metabolism that led to an increase in cholesterol.

The decrease in the level of protein and albumin might be linked with the impairment of the kidney or liver of Tor putitora. The decrease in the total proteins in the serum might be associated with the altered metabolism, decreased production of free amino acids or proteins, and elevation in the degradation of protein or proteolytic activity **(Ullah et al. 2021).** A significant decrease in the level of the globulin that observed by **Nisha et al. (2009)** in cattle and **Osman et al. (2009)** in fish that could be due to poor liver function and kidney damage due to heavy metals toxicity. Our results showed that the most polluted places were the more protein depleted fish for use in environmental pressures.

Pathological Study:

Accumulation of heavy metals affect fish vital organs where degeneration and necrosis of hepatocytes are recorded and attributed to accumulative effect of heavy metals in liver as mentioned by **Ashry et al. (2013) and Tayel et al. (2018)** who recorded that liver has an important detoxical role of endogenous waste products and toxins as heavy metals. Fatty change occurred in hepatic cells as result of metabolic disturbance due to heavy metals exposure **(Pacheco and Santos, 2002).** Presence of hepatic inflammatory cells as a result of a defense mechanism against the severe pathological changes induced by pb contamination, this cellular infiltration considered indicator for inflammatory response in fish as a result of toxicant presence **(Rezende et al. 2014).**

This study indicated the strong relation between heavy metals deposition and lesions in the liver. Similar alterations were reported by **Aly et al. (2003)** in cat fish Clariasgariepinusexposed to pollution by lead and confirmed by **Mustafa et al. (2020)** who mentioned similar alterations of liver tissue of Cyprinuscarpio and caraso-barbusluteus obtained from Tigris River.

Our results of pathological changes of kidneys are attributed to obvious blood supply of hemopiotic function of the kidney and in agree with **Omar et al. (2014)** who recorded that the highest metabolic activity organs such as liver and kidneys had the greatest ability to the highest levels of metals bioaccumulation.

Our findings in intestine are similar to the results obtained **by Fahmi et al. (2019)** and these damage and ulceration of intestinal villi lead to disruption in absorption process and growth process end reproduction leading to death .the damage of intestinal villi as a result of exposure to heavy metal which confirmed by **Underwood (2000)** who recorded damage of intestine caused by exposure to foreign objects which include excessive toxic damage in the body as mentioned by **Asri (2016)** who reported damage of intestinal epithelium and removal of intestinal villi and basal lamina propria in Dui fish intestine after contaminated with heavy metal . Chronic exposures to heavy metals lead to goblet cell hyperplasia aimed to sustaining life from toxix exposure to heavy metals. Goblet cells will synthesis and secret high molecular weight protein called mucin which increase mucosal surface protection against foreign exposure to heavy metals pollutions .

The pathological changes occurred in fish gills occurred as they the primary target organs for aquatic pollutants as mentioned by **Tchounwou et al. (2012) and Hermenean et al. (2017).**

Fonseca et al. (2017) mentioned that the impacts of metals could be of major importance and in correlation with filaments epithelium proliferation, lamellar fusion, and epithelial necrosis.

Lamellar vasodilatation observed could be attributed to increasing permeability caused by exposure to metals for long period which lead to necrosis and degeneration **(Mallatt, 1985).**

These mentioned changed might be consid-

ered as general defense response for increasing the space across which waterborne toxicants must diffuse for reaching blood vessels. Our results are similar to the results obtained by **Thophon et al. (2003)** mentioned several gill lesions in seabass. These alterations are in parrel with **Mustafa et al. (2020)** who mentioned similar alterations of gill tissue of Cyprinus-carpio and carasobarbusluteus obtained from Tigris River.

Metals level presence in the muscles which considered the main edible part in fish could threaten the public health. These results agree with those mentioned by **Taweel et al. (2013).**

In the present study, histopathologically alteration of the muscles attributed to heavy metal accumulation or changes in quality of water **(Osman and Kloas, 2010).**

CONCLUSION

The observations of our data showed that Pb was detected in variable concentrations in all samples, with the level of accumulation varying among African Catfish and Nile Tilapia fish from different farms. Hematological and biochemical parameters and pathological alterations indicated that heavy metals act as a stressor leading to changes in some blood parameters and accumulation in edible muscles and tissues. Potential adverse health effects in such application could be avoided if the wastewater is sufficiently treated.

Conflict of interest: none.

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