



(RESEARCH ARTICLE)



Pollution induced change of liver of *Oreochromis niloticus*: metals accumulation and histopathological response

Ibrahim Ahmed Thabet ^{1,*}, Wassif Ekbal Tawadrous ² and Alfons Mariana Samy ¹

¹ Zoology Department, Faculty of Science, New Valley University, Egypt.

² Zoology Department, Faculty of Science, Assiut University, Egypt.

Publication history: Received on 14 March 2018; revised on 12 June 2019; accepted on 18 June 2019

Article DOI: <https://doi.org/10.30574/wjarr.2019.2.2.0020>

Abstract

The present study, pointed to assess heavy metal accumulation like Aluminum (Al), Cadmium (Cd), Chrome (Cr), Cobalt(Co), Copper(Cu), Iron(Fe), Lead (Pb), Manganese(Mn), Nickel(Ni), Selenium(Se) and Zinc(Zn) in water, sediments and liver of *Oreochromis niloticus*, which collected from sewage water in El-kharja, New Valley, Egypt using inductively coupled plasma mass spectrometry (ICP-MS). Also, the histopathological changes of liver of *Oreochromis niloticus* were reported. These histopathological changes were detected using different staining types as a pollution biomarker. Iron showed the highest accumulation level in water (9.06±0.86 ppm), sediments (175.1±20.8 ppm) and liver (158.17±38.59 ppm), followed by Al> Mn> Ni> Zn> Pb> Cr> Se> Cu> Co> Cd. However, these elements showed Fe> Al> Ni> Mn> Cr> Cu> Zn> Pb> Cd> Se> Co accumulation trend in sediments. In liver these metals showed Fe> Se>Al> Zn>Mn> Cu>Ni> Cr> Pb> Co> Cd trend. Histopathological examination of fish liver showed signs of progressive alterations such as disorganization of architecture of liver cells, hydropic degeneration and vacuolation of hepatocytes. Also, dilation and congestion in blood sinusoids, hypertrophic and increase in number of küpffer cells were chronicled. Sever deposition of hemosiderin pigments were reported while necrosis with pyknotic nucleus and focal histopathological characters were observed side by side with normal cell. Our results concluded that water, sediment and liver accumulation toxicity tests and histopathological changes of liver may be associated, and these approaches may be used together to describe the environmental state and water quality assessment.

Keywords: Metal accumulation; Liver; Histopathology; Oreochromis; Sewage water

1. Introduction

The contamination of fresh water, with a wide range of pollutants has become a matter of concern over the last few decades [1-3], and is getting extensively contaminate metals released from domestic, industrial and other anthropogenic activities [4-8]. Metals are the main culprit for these undesirable changes in water quality [9].

Heavy metals exposure for a long time in very low concentrations caused histopathological and biochemical alterations in different tissues of fish [10]. Fernandes, Fontainhas-Fernandes [11], Mahboob, Al-Balwai [12] confirmed that, Cd, Pb and Hg are the most toxic metals, followed by Cu, Cr, Ni, Al, Mn and Zn; and the soluble forms of these metals are highly toxic to fish.

In polluted aquatic ecosystems, the histopathological alteration of organs is a tool to bioindicator of toxicant impact assessment to indicate fish health and toxicant effects. Also, these Histopathological alterations allow for speed detection of disease and detection of long-term injury in cells, tissues, or organs. Also the structural changes in many tissues in the contaminated ecosystem have also been approved [13]. Heavy metals accumulation, caused the formation of reactive oxygen species that caused changes in metabolism, further leading to cellular intoxication and

* Corresponding author

E-mail address: Ahmedt1983@scinv.au.edu.eg

death at a cellular level. This manifests as necrosis at the tissue level [14]. Mohamed [15], Thophon, Kruatrachue [16], Van Dyk, Pieterse [17] reported that the exposure of fish to pollutants like heavy metals resulted in several pathological alterations in different tissues of fish.

The liver of fish plays a vital role in basic metabolic functions and it is the most accumulated, biotransformed and excreted organ of toxins in fish [18]. Also, the histopathology of liver used as a bio-monitor for the environmental contamination [19]. Au [20], [21] reported that histopathological alteration of liver that exposed to heavy metals.

The present study was carried out in El-kharja city, New Valley Governorate, Egypt at El-shikh pond [22]. The quality of this ecosystem has been degrading due to industry, agriculture and human activities. Thus, the present study aimed to determine heavy metals accumulation in water, sediment and liver *Oreochromis niloticus* and histopathological alteration as a response to metallic pollution in liver as a bioindicator of the environmental quality of water, sediments and biota of the Lake.

2. Material and methods

2.1. Studied area and sample collection

It was carried out from April to July 2014 in El-shikh pond, which located between 25° 41' N to 25.43N and longitudes 30° 56' E to 30° 57' E at 4.33 Km extends. This pond receives untreated domestic sewage from numerous villages in addition to the agricultural and industrial wastes. One hundred and Fifty specimens of *Oreochromis niloticus* (23.85±10.29 cm) and (268.17± 124.11 gm) were collected in the same period. Fishes were transported to the laboratory in the containers with constant aeration. The wet weight and total body length of the fish were measured, blood sampling was done.

2.2. Heavy metals assessment

During the summer of 2014; water, sediments and fish liver samples were collected from the selected area. Water samples were collected from five localities in each of selected areas and different depth of water surface according to Boyd [23]. Five sediment samples were collected from 20 cm depth in polyvinyl chloride (PVC) corers [24]. Liver of fish were collected after fish minced and about 0.5 g was placed in a 100 ml beaker and 10 ml concentrated nitric acid was added for digestion and preparation. Heavy metals in water, sediment and fish liver were estimated using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) (Thermo Fisher Scientific, Bremen, GmbH).

2.3. Histology and histopathological examination

After liver collection of fish, immediately fixed in 10% Neutral formalin, then embedded in paraffin wax, passing through ascending grades of alcohol, clear in xylene, infiltrated using paraffin wax, embedded, mounted, sectioned at 3-5 µm using microtome (rotary) and subsequently stained with haematoxylin and eosin stain [25]. For the demonstration of the collagenous fibers Milligen's Trichrome stain used [26].

Polysaccharides, periodic acid Schiff's (PAS) technique were demonstrated using Mc Manus [27] technique. Mercury-bromophenol blue method was used to demonstrate the general protein, using Mazia, Drewer [28] method. Sections that stained were tested using Optica microscope and a digital camera.

2.4. Statistical analysis

The basic statistics (mean, standard error (SE),) were estimated using SPSS package release 10 [29].

3. Results

Table 1 The presented data (Mean \pm Std. Err.) of heavy metals accumulation of sewage water, liver and sediment of El-Shekh pond, undertaken during April-July 2014

* Heavy Metals	Water	EOS, 1993*	WHO, 2008#	Liver	EOS, 1993*	WHO, 2008#	Sediment
Aluminum (Al)	7.94 \pm 1.83*#	3	1	42.29 \pm 5.77#	50	30	173.9 \pm 3.8
Cadmium(Cd)	0.003 \pm 0.0004#	0.01	0.003	0.20 \pm 0.05#	0.5	0.05	2.04 \pm 0.02
Chromium(Cr)	0.20 \pm 0.01	1	0.5	1.31 \pm 0.15	20	10	4.85 \pm 0.05
Cobalt(Co)	0.01 \pm 0.002	0.2	0.2	0.25 \pm 0.04	10	1	0.25 \pm 0.01
Copper(Cu)	0.08 \pm 0.01	1	1	1.49 \pm 0.36	20	20	4.17 \pm 0.02
Iron(Fe)	9.06 \pm 0.86*#	0.3	0.3	158.17 \pm 38.59*#	30	30	175.1 \pm 20.8
Lead(Pb)	0.33 \pm 0.14*#	0.1	0.01	1.24 \pm 0.14#	2	0.05	3.43 \pm 0.05
Manganese(Mn)	0.90 \pm 0.24*#	0.1	0.1	3.67 \pm 0.75	10	10	124.4 \pm 5.2
Nickle(Ni)	0.82 \pm 0.03*#	0.1	0.1	1.41 \pm 0.19	10	2	150.2 \pm 7.3
Selenium(Se)	0.09 \pm 0.02	0.4	0.2	47.59 \pm 14.05	50	50	1.50 \pm 0.06
Zinc(Zn)	0.42 \pm 0.06	5	3	16.41 \pm 1.83	40	40	3.57 \pm 0.03

(*) More than EOS [30] permissible limits.

(#) More than WHO [31] permissible limits.

Agricultural and domestic wastes are the main indicators of chemical pollution that caused increases in heavy metals level in water resources. Metals accumulation data of water, liver and sediments are presented in table 1. Aluminum, Cd, Fe, Mn, Ni and Pb concentrations were more than permissible limits of EOS [30], and WHO [31] mentioning to the negative state of the studied ecosystems. Also, Fe then Al was the highest of metals concentration in sediments, on the other hand, was the lowest one.

Different patterns of heavy metal accumulations were observed in liver (Table 1). These patterns reflect the history and variety of fish in this aquatic ecosystem. This study showed accumulation of Al, Cd, Fe, and Pb were (42.29 \pm 5.77 ppm), (0.20 \pm 0.05 ppm), (158.17 \pm 38.59 ppm) and (1.24 \pm 0.14 ppm), respectively. These metals were more than the permissible limits of EOS [30] and WHO [31].

The normal structure of liver appears forming a meshwork and they arranged in a definite cord like pattern. The hepatocytes are polygonal in shape with an eccentric or centric spherical nucleus with a prominent nucleolus. Blood sinusoids separated these cells from each other (Fig. 1A). The defused pancreatic tissue surrounds branches of the hepatic portal vein within the liver. The exocrine portion consists of a large number of conical glandular cells. Each glandular cell has an eccentric, deeply stained nucleus with a prominent nucleolus. In the nuclear portion of the cell, the cytoplasm is homogenous and basophilic. On the other hand, the nature of the remaining part of the cell is acidophilic (Fig. 1B). Histopathological examination of fish liver showed signs of progressive alterations such as disorganization of architecture of liver cells, hydropic degeneration and vacuolation of hepatocytes. Also, dilation and congestion in blood sinusoids, hypertrophic and increase in number of K upffer cells were chronicled. Sever deposition of hemosiderin pigments were reported while necrosis with pyknotic nucleus and focal histopathological characters were observed side by side with normal cell (Fig. 2). Examination of the hepatopancreatic tissue showed histopathological alterations such as shrinkage and disarrangement of pancreatic acinus. Vacuolation in pancreatic cells and adipose tissue were noticed between pancreatic cells. Increase in edema and thickening in the wall of portal vein (Figs. 3). Milligen trichrome stain revealed a great amount of connective tissue (fibers and adipose tissue) around the portal vein and between pancreatic acini respectively, also in hepatic tissue (Fig. 4).

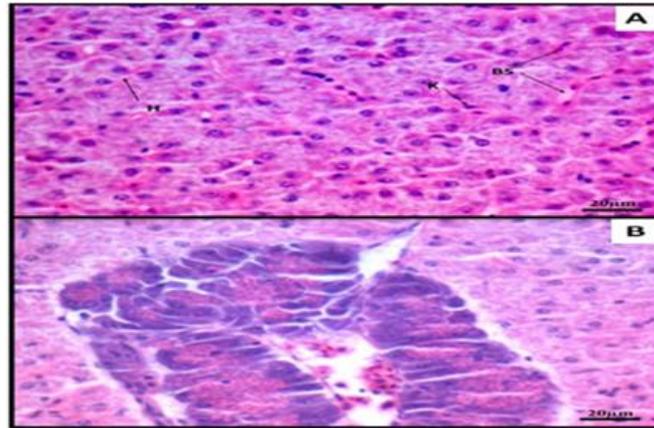


Figure 1 Photomicrograph of fish normal liver showing the general structure (A): Blood sinusoids (BS), hepatocytes (H) and Kupffer cell (K) (B): portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. (H&E, X 400).

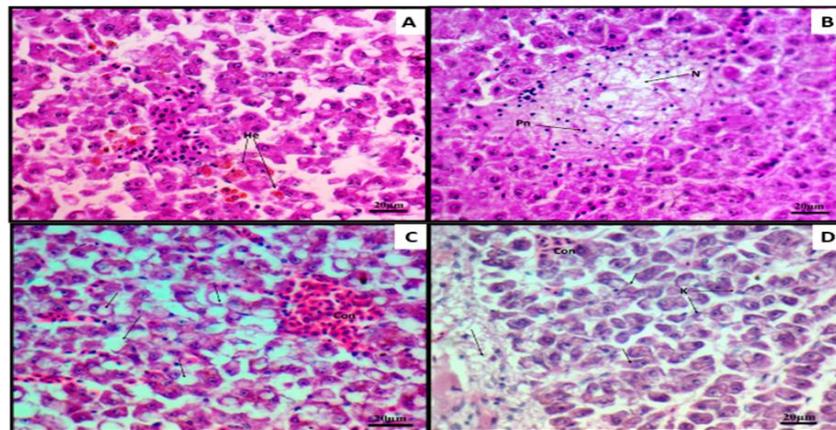


Figure 2 Photomicrograph of fish liver showing (A): hemosiderin granules (He), (B): necrotic area (N) with pyknotic nuclei (Pn), (C): fatty degeneration (arrows) blood congestion (Con) and (D): blood congestion (Con), acute massive necrosis, shrinkage and dissociation of cell (arrows) and hypertrophy of Kupffer cell (K). (H&E, X 400)

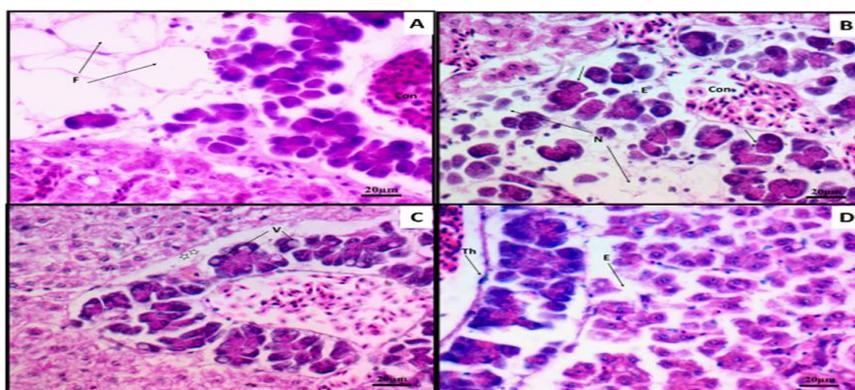


Figure 3 Photomicrograph of fish hepatopancreas showing (A): adipose tissue (F) between pancreatic cells and blood congestion (con), (B): blood congestion (Con), edema (E), necrosis (N), shrinkage and disarrangement of pancreatic acinus (arrows), (C): space between liver and pancreas (stars), vacuolation in pancreatic cells (V) and (D): thickening wall of portal vein (Th), edema between hepatocytes (E) and disarrangement of hepatocytes. (H&E, X 400).

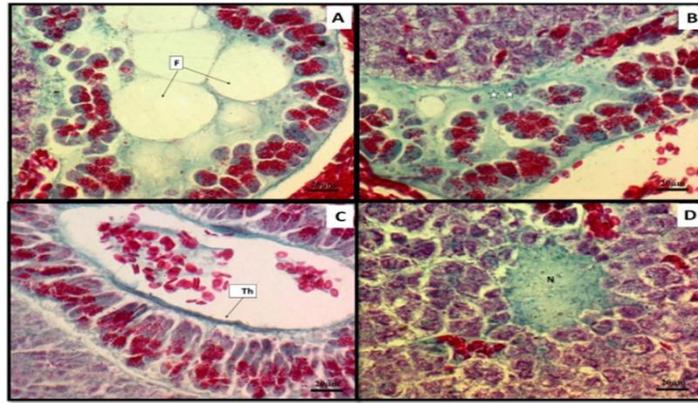


Figure 4 Photomicrograph of fish hepatopancreas showing (A): adipose tissues (F) between pancreas cells, (B): increase of fibrous connective tissues (stars), (C): thickening (Th) around portal vein and (D): necrotic area (N) between hepatocyte. (Milligen Trichrome, X 400).

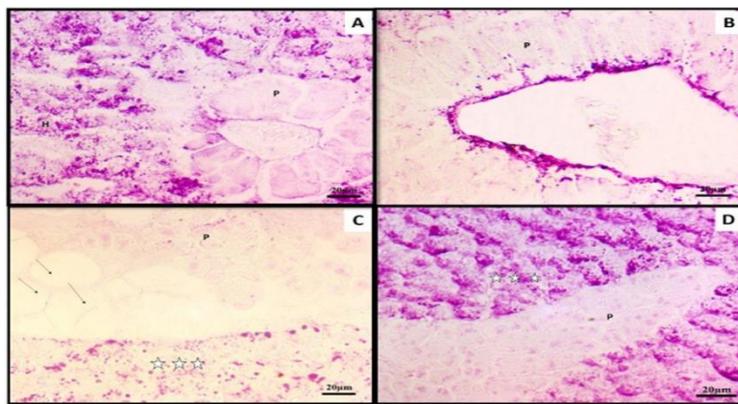


Figure 5 Photomicrograph of fish normal liver showing (A): glycogen content in hepatocytes (H), hepatopancreas (P) tissue, (B): thickening around in portal vein, (C): fatty infiltration between pancreas cells (arrows) and depletion of glycogen contents in hepatocytes (stars) and (D): negative reaction for pancreas cells (P) and increment of glycogen contents in hepatocytes (stars). (PAS-reaction, X 400)

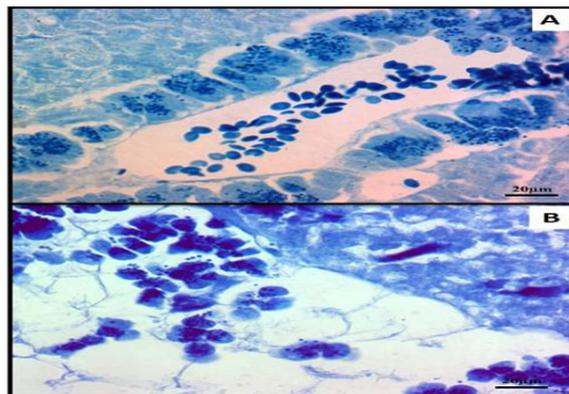


Figure 6 Photomicrograph of fish normal hepatopancreas showing (A): zymogenic granules within pancreatic portion. Equivalent to acidophilic portion of cells and (B): adipose tissue between pancreas cells and liver. (Bromophenol Blue stain, X 400).

PAS reaction revealed a high fluctuation of glycogen content in liver tissue. The negative PAS reaction was noticed in hepatopancreatic tissue, while the positive PAS reaction was detected near the portal vein (Figs. 5). Using bromophenol blue to identify general proteins, the cytoplasm of hepatocytes in normal tissue was faintly stained but

the zymogen granules were strongly stained. In alter tissue both hepatocytes and hepatopancreatic acini were strongly stained (Figs. 6).

4. Discussion

The Using of fertilizers in agricultural uprising caused increases of heavy metals accumulation in fresh water due to the water run-off, which caused metallic pollution [32, 33]. In this respect, Abou Elnaga and Allam [34] reported that heavy metals bioaccumulation in different tissues of fish body not similar to its concentration in the ambient water. Also, the low concentration of heavy metals in water may cause high concentration of heavy metals in fish tissues [35]. Fish are exposed to pollutants through two pathways (direct and indirect); up taking from water, through food or both. The direct uptake depends on the total concentration and the bioavailability of the pollutant, like the physiological factors of fish [36].

Our results showed that Al, Fe, Mn, Ni and Pb concentrations were more than permissible limits of EOS [30], and WHO [31] mentioning to the negative state of the studied area. Sediment showed that Fe then Al were the highest metals in concentration. However Co than Se was the lowest one. Similar to our results, Omar, Zaghoul [37] and Ibrahim, Wassif [22] who found highly precipitation of heavy metals concentration from water column sediment samples.

The present investigation showed that Al, Cd, Fe and Pb concentrations in liver of *O. niloticus* were more than the permissible limits of EOS [30], and WHO [31]. Similarly, Authman, Abbas [38], Ibrahim [39] and Ibrahim, Wassif [22] found that these elements are more than permissible limits in liver of *O. niloticus*. Also, Kaoud and El-Dahshan [40] reported that Cd and Cu concentrations were the highest in liver. The high Zinc accumulation in liver was in agreeing with Ibrahim [39], El-Naggar and Tayel [41], Ibrahim and Omar [42]; Ibrahim, Wassif [22]. Ibrahim [39] also, found that Fe and Ni accumulation in liver as a target organ of *Oreochromis niloticus*.

Liver play as multiple oxidative reactions site that formed from metal accumulation and maximal free radical generation [22, 39, 42]. Metallic pollution caused the formation of free radicals in different tissues which caused histopathological alteration in different tissues [43]. Liver is the detoxification organ of fish; and its high tendency of liver to accumulate heavy metals more than other organs caused the formation of complexation of metal ions, which leads to DNA fragmentation [44].

The normal structure of liver appears forming a meshwork and they arranged in a definite cord like pattern. The hepatocytes are polygonal in shape with an eccentric or centric spherical nucleus with a prominent nucleolus, which separated from each other by blood sinusoids. Fayed [45] reported that hepatic vein received blood from the hepatic portal vein and hepatic artery through the sinusoids to the central veins. The defused pancreatic tissue surrounds branches of the hepatic portal vein within the liver. The exocrine portion consists of a large number of conical glandular cells. Each glandular cell has an eccentric, deeply stained nucleus with a prominent nucleolus.

The present study showed a histopathological alteration of liver like hydropic degeneration, vacuolation of hepatocytes, dilation and congestion in blood sinusoids, hypertrophic and increase in number of K upffer cells. Also, hemosiderin pigments were reported while necrosis with pyknotic nucleus and focal histopathological characters were observed side by side with normal cell. Examination of the hepatopancreatic tissue showed histopathological alterations such as shrinkage and disarrangement of pancreatic acinus. Vacuolation in pancreatic cells and adipose tissue were noticed between pancreatic cells. Increase in edema and thickening in the wall of portal vein. Similar alterations were observed in the hepatocytes of *Clarias gariepinus*, *Tilapia zillii* and *Solea vulgaris* living in contaminated areas with endocrine disrupters and heavy metals [46-48]. *Oreochromis niloticus* exposed to heavy metals in its environment and laboratory display the same histopathology [21]. The vacuolization of hepatocytes in the liver was a more common pathology in the fish exposed to contaminants in their environments [49, 50] and is associated with the inhibition of protein synthesis, energy depletion, and desegregation of microtubules, or shifts in substrate utilization and accumulation of lipid responses to toxic substances. [51] suggesting that vacuolization might be the result of the chemical substance exposure. Oxygen deficiency as a result of gill degeneration and/or to vascular dilation and intravascular hemolysis reported in blood vessels with subsequent stasis of blood cause of the cellular degeneration in liver [52]. The increasing of K upffer cells in size and number were as a defense mechanism against stress and foreign material in the blood circulation [53]. These alterations were also described under the effect of herbicide or heavy metals on *Oreochromis niloticus* and catfish *Chrysichys auratus* in the laboratory [54-56]. Similar results were observed by Triebkorn, Telcean [57], Van Dyk, Pieterse [58] who reported similar histopathological alterations in liver of fish that exposed to different metals.

The abnormal accumulation of hemosiderin in liver may be due to many factors like, rapid destruction of RBCs by conversion of hemoglobin into hemosiderin and damage of Fe metabolism [46, 59]. Also, high amount of Fe accumulation in fish liver, leading to abnormal accumulation of hemosiderin [59]. Khan [60] also, reported a strong link between hemosiderin pigment formation and hepatic alteration.

The present staining with Milligen trichrome showed a great amount fibers and adipose tissue as a connective tissue, which were found around the portal vein and between pancreatic acini respectively, also in hepatic tissue. Also, the fatty degeneration alteration in liver may be due to a decrease in the rate of utilization of energy reserve or pathological enhance synthesis, and abnormal accumulation of fats was reported in experimental animal, which formed due to induced imbalance between fat production and utilization [61]. The hepatic necrosis results from pollutant within cells, causing disturbs on biochemical process as enzyme inhibition, failure on protein synthesis and carbohydrate metabolism [62, 63].

Acute massive necrosis of liver were noticed in the present investigation. Sandritter and Thomas [64] described this alteration as a disorganization of liver cell cords, that is, the individual liver cells have become unattached and appear as separated cellular elements. The cells vary in size, and some are already shrunken. The cytoplasm is homogenous and stains more blue than normal (decrease in glycogen content). The nuclei more faintly stained than normal (karolysis) (Fig. 5D).

Similar to our findings, fish inhabiting areas contaminated with different types of pollutants such as heavy metals [21], waste water treatment plant effluent [38] and laboratory experiments [55] displayed histopathology alterations on their liver tissues.

The high fluctuation of glycogen content in liver was revealed due to PAS reaction as a negative reaction in hepatopancreatic tissue, while a positive reaction was detected near the portal vein. Hepatopancreas showed an inhibition of acidity of the apical portion of pancreatic acinar cells. Wassif, Kider [56] reported similar results when exposed of *Oreochromis niloticus* to different pollutants. Cellular proliferation observed here in pancreatic tissues could possibly explain by the stimulatory effect that is originated from striking deterioration of liver cells. Enhanced fibrosis, i.e., High proliferation of CT, of the examined organs resulted in marked inhibition of the various vital activities [19].

Using bromophenol blue to identify general proteins, the cytoplasm of hepatocytes in normal tissue was faintly stained but the zymogen granules were strongly stained. In alter tissue both hepatocytes and hepatopancreatic acini were strongly stained. The present study showed a notable collection of connective tissue fiber adjacent to some blood vessels in liver. Similar results were observed by Blazer [65] who found fibrosis and suggested to be a chronic tissue response to chemical injury.

5. Conclusions

The metallic pollution in the selected area, with high metal concentrations that recorded in water, sediment samples, because of the continuous discharge to the aquatic habitats. It confirms that it is having a strong impact on fish health as these heavy metals are accumulating in liver of *Oreochromis niloticus*. Application of metal detection in liver of *Oreochromis niloticus* and histopathological alteration, provides valuable biomarkers in field surveys, in monitoring studies and in comparing different levels of metallic pollution. Severe histopathological lesions and cellular alterations were observed in fish liver, which could be attributed to the significant accumulation of several heavy metals in liver, which, used as a sensitive model to monitor the aquatic pollution. That heavy metals accumulation reached to a dangerous level that affecting the health of local human communities.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science, New Valley University, Egypt.

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How to cite this article

Ibrahim AT, Wassif ET and Alfons MS. (2019). Pollution induced change of liver of *Oreochromis niloticus*: metals accumulation and histopathological response. World Journal of Advanced Research and Reviews, 2(2), 25-35.
