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Effects of heavy metal pollutants on the reproduction of Nile tilapia

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Abstract
Many farms surrounding Lake Manzala near the Suez Canal area suffer from severe environmental pollution. The source of pollution in that area comes from different sources of industrial, sewage, agricultural activities and petroleum companies which present in that area. This research aims to study the effects of some environmental pollution especially heavy metals at these locations of Lake Manzala on the *Oreochromis niloticus* reproduction. There were two groups, one was an intoxicated group (50 fish) and the other one was 50 fish from Abbasa farm as a control group. Fish were transported to the laboratory for studying clinical signs, histopathological examination and detection of heavy metals accumulation in fish organs which involved in reproduction as (liver, brain, ovary, testes). The results showed that there was a significant difference between the intoxicated group and the control group. The metals levels were Fe >Cu>Pb>Cd respectively. The accumulation of (Fe and Cu) were in liver>brain>ovary>testis respectively. While Pd was accumulated in liver >brain> ovary> testis respectively. While Cd was accumulated in liver >brain>testis>ovary respectively. The histological study of these organs (Ovary, testes, liver and brain) was recorded. The testis of the fish showed some degenerative changes and decreased number of seminiferous tubules. They appeared empty from different spermatogenic cells. The ovaries showed deformation from their normal shapes and sever lymphocytic infiltration. The liver showed necrosis and aggregation of melanomacrophages (MMCs). The brain also showed degeneration of neurons, severe loss of granular cells and vacuole formation. We recommended improving the quality control of water and environmental monitoring is necessary for these areas.

Keywords: Heavy metals, tilapia, pollution, histopathology

1. Introduction
Fish play an important role in the assessment of pollution potential risks [1, 2]. Fish which exposed to pollutants especially heavy metals such as (Cd, Fe, Pb, and Cu) may suffer from acute or chronic toxicity which affects all physiological processes including reproduction. The healthy reproductive process of fish is an important indicator of the fish to be able to sustain itself [3]. People prefer to fish in their food as it has a palatable taste and economic price. Fish is the most important and inexpensive source of animal protein [4, 5].
Lake Manzala is the most important lake in Egypt. It has boundaries with the Suez Canal area and Port Said. These areas are the most polluted area due to petroleum waste and Industrial effects [6]. At the same time, it produces about half of the production of fish in Egypt [7]. Histopathological alteration or cellular changes in tissues such as liver, brain, ovary, and testes have received much attention in assessment of the effects of environmental pollution [8-10]. Historically, there is no more attention to endocrine, neural and gonadal histology; all systems that affect reproduction. There are many environmental chemicals that act as endocrine disruptors and affect fish reproduction, this led to an interest in assessing the reproductive health of fish [11-14]. Water pollution happens when pollutants discharged directly or indirectly into lakes, rivers, etc. without the removal of harmful substances. Water pollution affects fish and other organisms living in this water [15]. The environmental pollutants especially heavy metals may increase the incidence of diseases by reduction of immune state, reproductive and developmental processes of the organism [16]. The present research aimed to study the histopathological effects of different heavy metals on freshwater fish gonads of Nile tilapia, *O. niloticus* and determination of the level of these metals (Fe–Cu–Pb–Cd) in these organs (liver –brain-ovary-testis) and water analysis where the fish was lived.

2. Materials and Methods

2.1 Studying area

A private aquaculture farm in the Suez Canal area at the Northeast of lake Manzala which affected by petroleum compounds.

2.2 Fish

One hundred Nile tilapia fish *O. niloticus* (200 ± 10 g mean body weight*)* 50 of them from the polluted studied area and 50 from Abbasa farm as a control group.

2.3 Water analysis

Lake Manzala polluted water and Abbasa farm water as control were collected in a dark glass bottle and sent to the lab.

3. Methods

3.1 Experimental Design

100 Nile tilapia fish *O. niloticus* (200 ± 10 g mean body weight) were collected a life from a private aquaculture farm and Abbasa farm then transported life to the laboratory. The first weight and the total length of the examined fish were recorded.

3.2 Chemical analysis of water

Heavy metals (Copper, cadmium, lead, iron), were measured using an atomic absorption spectrophotometer.

3.3 Tissue sampling

Tissue specimens from the liver, brain, gonads were dissected into two parts one for histopathological studies and the other for determination of the accumulation of heavy metals in tissues by the atomic absorption spectrophotometer

3.4 Histopathological studies

To study the histopathology of gonads, liver, and brain. Adult samples of *O. niloticus* were collected from the studied area and brought alive to the laboratory. After dissecting the fish organs (Testes, ovaries, liver, and brain) were immediately removed and fixed in formalin 20% solution for 24 h. The tissues were routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6 µm thick were cut, processed and stained with hematoxylin and eosin (H and E) and then, Canada balsam was poured and covered with a cover glass. They were examined and photographed under a light microscope unit [17, 18].

4. Results

1. Clinical signs

Fig 1: Fish showed hemorrhage, ocular problem, weight reduction and ulcers (photos a & b). PM lesions showed a pale color of liver, petechial hemorrhage, and severe congestion (Photos c & d).

Table 1: Metals concentration (ppm) in the water in Manzala and Abbassa farm as control (mean ± S.E).

Heavy Metals	Manzala water	Abbasa water	The permissible limit of Egyptian Standardization
Fe	1.8	0.25	>0.300
Cu	1.43	0.16	1.0
Pb	0.11	0.09	0.1
Cd	0.035	0.01	0.01

Table 2: Metals concentration (ppm) in the liver in Manzala and Abbassa farm as control (mean ± S.E).

Group Test	Toxicated Group	Control Group	Sig.	ETA	ETA^2
Fe	2338.740 ± 290.103	166.446 ± 21.407	0.000 *	0.935	0.875
Cu	157.924 ± 21.523	14.463 ± 0.823	0.000 *	0.920	0.847
Pb	11.862 ± 2.335	0.451 ± 0.151	0.001 #	0.865	0.748
Cd	6.030 ± 1.223	0.187 ± 0.028	0.001 #	0.860	0.740

The mean difference is significant at the (*: P<0.001, #: P<0.005) levels.

Table 3: Metals concentration (ppm) in brain in Manzala and Abbassa farm as control (mean ± S.E).

Group Test	Intoxicated Group	Control Group	Sig.	ETA	ETA^2
Fe	349.912 ± 45.647	83.056 ± 6.764	0.000 *	0.898	0.807
Cu	57.330 ± 3.577	9.968 ± 0.830	0.000 *	0.977	0.954
Pb	4.501 ± 0.941	0.400 ± 0.081	0.002 #	0.838	0.702
Cd	2.996 ± 0.414	0.049 ± 0.004	0.000 *	0.929	0.864

The mean difference is significant at the (*: P<0.001, #: P<0.005) levels.

Table 4: Metals concentration (ppm) in ovary in Manzala and Abbassa farm as control (mean ± S.E).

Group	Intoxicated Group	Control Group	Sig.	ETA	ETA^2
Fe	231.091 ± 19.280	81.504 ± 4.677	0.000 *	0.936	0.877
Cu	23.359 ± 3.436	12.398 ± 1.543	0.020 #	0.717	0.514
Pb	2.700 ± 0.289	1.593 ± 0.270	0.029 #	0.719	0.517
Cd	0.505 ± 0.101	0.195 ± 0.050	0.039 #	0.690	0.477

The mean difference is significant at the (*: P<0.001, #: P<0.05) levels.

Table 5: Metals concentration (ppm) in testis in Manzala and Abbassa farm as control (mean ± S.E).

Group Test	Intoxicated Group	Control Group	Sig.	ETA	ETA^2
Fe	134.863 ± 22.385	57.798 ± 5.472	0.010 *	0.764	0.583
Cu	10.386 ± 1.568	6.314 ± 1.059	0.064	0.606	0.367
Pb	2.659 ± 0.266	1.755 ± 0.245	0.045 *	0.678	0.459
Cd	1.400 ± 0.281	0.620 ± 0.323	0.110	0.569	0.323

The mean difference is significant at the (*: P<0.05) level.

4. The histopathological results of selected organs as the following

4.1 Testis

Histopathological examination of the testis of the polluted group demonstrated that the testis of Nile tilapia showed loss of its ideal shape of seminiferous tubule and has residuals of primary and secondary spermatocytes. Testes display severe congestion in interstitial capillaries, Fig (3a) while histopathological findings in the control group are

4.2 Ovaries

Microscopically, the ovary of Nile tilapia showed severe lymphocytic infiltration, Fig (3 b). According to our study, the gonads of *O. niloticus* are obviously suffering from deformation from their ideal shapes.

4.3 Liver

As shown in Fig (3 c) liver displayed necrosis of hepatocytes and hepatopancreas. There are sever lymphocytic infiltrations and necrosis.

4.4. Brain

The histological section in the brain illustrated severe congestion (arrow), edema in the brain parenchyma and glial cell proliferation, Fig (3d).

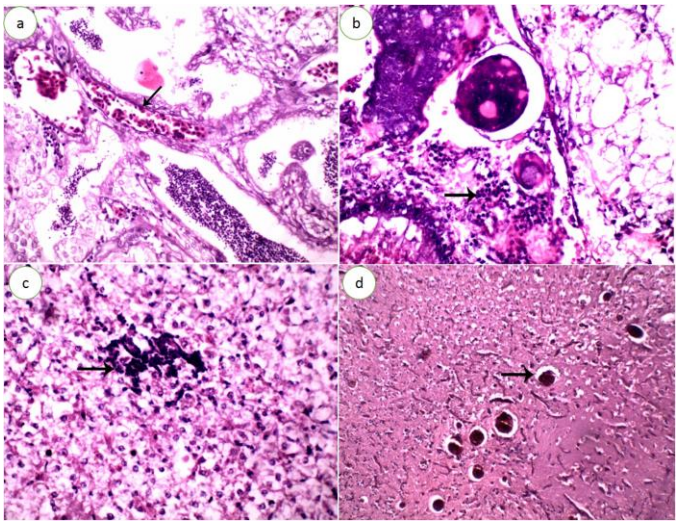

Fig 2: Normal tissues of Nile tilapia; testis (a), ovaries (b), liver (c), and brain (d).

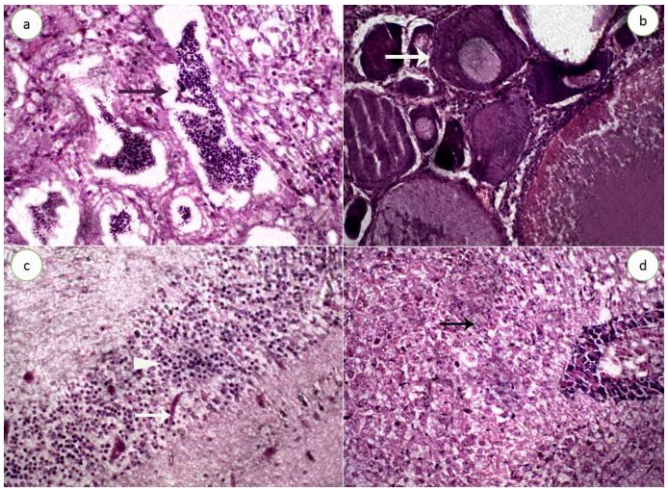

Fig 3: Tissues of intoxicated Nile tilapia; testis (a), ovaries (b), liver (c), and brain (d).

Discussion

Mekkawy and Hassan [19] and Ojaveer *et al.* [20] stated that the reproductive functions are a key factor in determining the dynamics of fish populations. The presence of pollutants is one of many environmental problems that can result in a dangerously compromised fish reproduction system. Many studies have proven that exposure to pollutants especially heavy metals can lead to a decrease in gonad somatic index [21], dysfunctional hormone reproduction [22], shrinking oocyte diameter [23], alteration in reproductive behavior [24] and increased abnormalities of fish larvae [25].

Nofal and Abdel-Latif [6] have stated that in Egypt, at Manzala, the environmental pollution of the aquatic fish system seems to be a serious devastating problem. In the past 10 years, Khare and Singh [26] found that more toxic compounds are being detected in the aquatic fish ecosystem. El-Morshedi *et al.* [27] reported that the environmental pollution of Lake Manzala caused a great decline in gonad activity of the studied fish, where was reflected in decreasing sperm in ripe testes and ripe oocytes degeneration (atresia). Also, the sexual development disruption, inducing masculinization and severe pathological alterations in testis, such as the enlargement of sperm ducts, interstitial changes, and basal membrane detachment, while there were atretic oocytes in ovaries [28].

Water pollution has a serious inhibitory effect in fish reproduction [29]. The different pollutants such as heavy metals, industrial wastes, pesticides, and agricultural wastes,

and different types of bacteria have histopathological effects on the reproductive tissues of fish gonads [30, 31]. The maximum allowance levels of lead and cadmium in surface water were 0.05mg/l and 0.01mg/l respectively [32]. WHO [33] stated that the standard of Iron is 0.2 mg/L and copper limit is of 2.0 mg/L. The US Environmental Protection Agency (USEPA) developed a health-based action level of 1.3 mg/l Cu in drinking water [32] and an aesthetic-based standard of 1 mg/l Cu. On comparing our results, we can find that the levels of cadmium, lead, iron, and copper in a water sample are higher than the permissible limits and Abbasa farm which acts as a control group, so constitute a potential health hazard.

Also, these results were matching with Saeed and Shaker [34] who said that, in Lake Manzala, metals had the sequence of Fe > Cu > Zn > Pb > Cd. Additionally, it has been also matched with Elghobashy *et al*. [35] said that Fe> Cu>Pd>Cd in lake Manzala. This order of occurrence agrees with the previous studies performed on Lake Manzala [36]. Also matched with Ali [37] who found that Fe >Cu >Pd >Cd, and these results disagree with Zahran *et al.* [38] who found that Fe >Pd >Cu>Cd.

The gonads deformation of *O. niloticus* from their ideal shapes was found to indicate the effect of the pollution on the different regions of Lake Manzala. The deformations of testis and ovary at the studied site were obviously noticed. This is maybe due to the presence of petroleum Companies near to El-Gmail site, or the presence of many kinds of pollutants (sewage, industrial, and agricultural). In this research, the results of gonads histological examination sharply proved that the pollution especially by heavy metals had their great effect on the gonads of *O. niloticus and so the reproduction*. Its effect appeared as a gonadal development disruption. It agrees with Al-Halani [39] study for fish inhabiting polluted water.

The different pollutants such as heavy metals and industrial and agricultural wastes, pesticides have histopathological effects on the reproductive tissues of fish gonads [40, 41]. These effects may upset the development of germ cells and may decrease the ability of the fish to reproduce [42, 43]. Also, the testis of the studied fish collected from the polluted water in the studied area showed an impairment of spermatogenesis and lobular structures and the suppressing sperm production was observed by former studies [17, 31]. The effects of pollutants especially heavy metals on fish reproduction have been inspected by many researchers who confirmed adverse effects on fish organs and especially their reproductive capacities. For example, Molina *et al.* [44] reported that follicular atresia is caused by exposure to pollutants. A similar study carried out by Senarat *et al.* [45] also confirmed the relationship between pollutants and the increase of follicular atresia, which was caused by the lowering of activities of gonadotropin hormone (GTH) and other estrogen hormones. Sridevi *et al.* [46] reported that the disturbance that occurred in the reproductive hormone led to a decrease (degeneration) of yolk size. Patiño and Sullivan [47] cleared that oocytes contain different regulatory compounds such as different lipophilic hormones and vitellogenin receptors, steroids, enzymes, antibodies, and different other active Compounds. Exposure to pollutants especially heavy metals leads to dysfunction of the reproductive hormone which then affects the vitellogenin receptors, i.e. oocytes disruption [48].

Conclusions

The results of the histological examination of gonads and water analysis showed that the pollution, especially by heavy metals, had their major effects on the gonads of *O. niloticus*. Also, the present research documented that increasing water quality control and periodically environmental monitoring are needful for Lake Manzala water. In Egypt, although there is a marked decrease in pollution content in Lake Manzala when compared to the last 10 years, more governmental efforts still needed to control environmental pollution and ameliorate its water quality.

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