Bioaccumulation of Some Heavy Metals and Histopathological Alterations in Liver of Oreochromis niloticus in Relation to Water Quality at Different Localities along the River Nile, Egypt

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Abstract: Fish samples were collected from the River Nile and its two branches (Damietta and Rossetta). The sampling stations were El-Oqsur, El-Menia, El-Hawamdia, Shoubra El-Khema, El-Rahawy drain, Kom Hamada, Talkha, El-Serw and Faraskour. The accumulation of Iron, Manganese, Copper, Zinc, Cadmium and lead in liver of collected O. niloticus fish from the above stations were determined. In addition, the same liver samples were examined histopathologically. Results showed that trace metals accumulations in fish liver at area under investigation were detected in following descending order: Fe > Cu > Zn > Mn > Pb > Cd. Histological study indicated that the liver of O. niloticus living in the studied stations showed several pathological alterations including: degeneration, fatty degeneration, necrosis and edema. Also congestion, branching (anastimosis), hemorrhage, hemolysis, hemosidrin and parasitic forms were seen in blood vessels. It was noticed that the liver of fish collected from Shoubra El-Khema, El-Hawamdia, El-Rahawy drain and El-Menia showed much more damages than that collected from the other stations as these sites receives more drainage water loaded with industrial and sewage wastes than the other stations. It was concluded that the discharge of different types of wastes, especially heavy metals deteriorated the water quality in the River Nile and consequently affecting fauna and fish production and human health. It is recommended to treat the different wastes before discharging to the River Nile Stream.

Key words: River Nile %Heavy metals %Liver %Oreochromis niloticus %Histopathology

INTRODUCTION

The River Nile is the principal fresh water resource and life for Egypt representing more than 97% of Egypt’s water resources [1, 2]. As a sequence of increasing industry, agriculture urbanization and tourism, human activities are responsible for chemical pollution sources for the environment and aquatic ecosystems [3, 4].

According to National Water Research Center [5], the River Nile from Aswan to El-Kanater Barrage receives wastewater discharge from 124-point sources, of which 67 are agricultural drains and the remainders are industrial sources.

Along Damietta branch, there are Talkha fertilizer plant, Kafer saad electric power station, Delta milk, Edfina factories, besides the sewage and domestic wastes discharging from the neighboring villages along Damietta. (El- Serw and Ras El-Bar cities) without any treatment into the branch [6, 7].

The River Nile at El-Rahway drain receives all sewage of El-Gieza governorate in addition to agricultural and domestic wastes of El-Rahway village and discharged these wastes directly without treatment into Rosetta branch [8].

Heavy metals have a great ecological consideration due to their toxicity and accumulation [9]. Fish may accumulate significant concentrations of metals in water in which those metals are below the limit of detection in routine water samples [10]. The impact of heavy metals on aquatic environment affects directly or indirectly human health [11]. Some heavy metals such as copper and iron are essential for growth and well-being of living organisms including man. However, they likely show toxic effects when organisms are exposed to higher doses than those normally required. Other elements such as Pb and Cd are non-essential for metabolic activities and exhibit toxic properties even with trace level [12].
The histological studies are considered as direct evidence referring to any adverse effect on fish. Generally, the liver is considered as the principal organ of detoxification in vertebrates and particularly in fish. It is also the potential site for lipid deposition in these animals [13]. Meanwhile, fish liver is a good indicator of aquatic environmental pollution, where one of the important functions of the liver is to clean of any poisons or pollutants from the blood coming from the intestine [14].

The present study aimed to evaluate the pollution status of some areas along the River Nile, Egypt using Oreochromis niloticus liver biochemistry and histology as biomarkers due to it is highly esteemed fish in Egypt.

MATERIAL AND METHODS

Sampling Stations: Nine stations were selected along the River Nile and its two branches covering the different environmental conditions of the River Nile. These stations were El-Oqsur, El-Menia, El-Hawamdia, Shoubra El-Khema, El-Rahawy drain, Kom Hamada, Talkha, El-Serw and Faraskour Fig. 1.

Fish Samples: Samples were collected from O. niloticus during summer 2008 from the selected nine stations to carry out the following study.

Biochemical Analysis: A representative sample of 1g dry weight of each liver sample was taken from fish specimens. These samples were digested according to the method described by Goldberg et al. [15] in which concentrated nitric and perchloric acids with ratio of 5:5 ml were used in Teflon beakers on a hot plate at 50 °C for about 5 hours till complete decomposition of organic matter. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50 ml using deionized distilled water. The concentration of iron, manganese, copper, zinc, cadmium and lead in liver of the selected fish were measured by Atomic Absorption (I.C.P. plasma 400). Results were expressed in µg/g dry weight of the tissue.

Histopathological Analysis: Liver samples from each collected fish were carefully removed and fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue were embedded in paraffin wax and sectioned into five micrometres thick, then stained with hematoxylin and eosin method according to Bucke [16]. Then the sections were examined on light microscope and photographed by using a microscopic camera. The fish of the control group was collected from a fish farm in El-Kanater El-Khyria which is out of pollutants

Statistical Analysis: Statistical analysis was carried out using Student “t” Test to compare between the different results of the heavy metals and parasite infections (SPSS, 16)

RESULTS

Bioaccumulation of Heavy Metals: The accumulation of Iron, Copper, Zinc, Manganese, Lead and Cadmium in liver of O.s niloticus obtained from the nine studied stations were shown in Table 2.

The present data show that trace metals accumulations in fish liver at area under investigation were detected in the following descending order: Fe > Cu > Zn > Mn > Pb > Cd.

The highest values of Fe, Cu, Zn and Mn recorded at site 4, which received Industrial wastes while the lowest values of these metals recorded at site 1, which received agricultural wastes.

The lowest values of Pb and Cd were recorded at site 3 and the highest values of these elements were recorded at site 6 and site 5, respectively.
Table 1: The sampling location along the River Nile (biforked, Dametta and Rosetta branches)

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>El-Oqsur 1</td>
<td>228 km from High Dam (beside El-Karnak)</td>
</tr>
<tr>
<td>El-Menia 2</td>
<td>677 km from High Dam before El-Menia</td>
</tr>
<tr>
<td>El-Hawamdia 3</td>
<td>912 km from High Dam in front of the sugar and integrated industries company</td>
</tr>
<tr>
<td>Shoubra El-Khema 4</td>
<td>After the electric station</td>
</tr>
<tr>
<td>El-Rahawy drain 5</td>
<td>In front of the drain</td>
</tr>
<tr>
<td>Kom Hamada 6</td>
<td>In front of the Mohamed island</td>
</tr>
<tr>
<td>Talkha 7</td>
<td>126 km north of Cairo after Talkha power station and fertilizer factory</td>
</tr>
<tr>
<td>El-Serw 8</td>
<td>170 km north of Cairo at Serw City</td>
</tr>
<tr>
<td>Faraskour 9</td>
<td>210 km north of Cairo behind the Faraskour Dam</td>
</tr>
</tbody>
</table>

Table 2: The residual analysis of trace metals in liver of Oreochromis niloticus tissue (μg/g dry weight, Mean±SE)

<table>
<thead>
<tr>
<th>Station</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>El-Oqsur</td>
<td>94.5±0.23</td>
<td>18±0.04</td>
<td>12.2±0.10</td>
<td>7.5±0.09</td>
<td>7.38±0.07</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>El-Menia</td>
<td>215.32±0.22</td>
<td>45±0.55</td>
<td>25.5±0.20</td>
<td>19.5±0.17</td>
<td>7.3±0.08</td>
<td>0.8±0.01</td>
</tr>
<tr>
<td>El-Hawamdia</td>
<td>300.2±0.42</td>
<td>33.2±0.43</td>
<td>25.1±0.18</td>
<td>25.01±0.03</td>
<td>3.9±0.02</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Shoubra El-Khema</td>
<td>820.3±0.34</td>
<td>55.7±0.21</td>
<td>30.4±0.32</td>
<td>30.3±0.22</td>
<td>5.1±0.04</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>El-Rahawy drain</td>
<td>230.8±0.23</td>
<td>53.8±0.22</td>
<td>26.1±0.33</td>
<td>17.5±0.03</td>
<td>8.1±0.06</td>
<td>1.3±0.02</td>
</tr>
<tr>
<td>Kom Hamada</td>
<td>192.8±0.80</td>
<td>22.1±0.17</td>
<td>21.2±0.12</td>
<td>11.2±0.34</td>
<td>10.5±0.09</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>Talkha</td>
<td>150.7±0.30</td>
<td>21.5±0.21</td>
<td>20.4±0.22</td>
<td>9.8±0.14</td>
<td>9.6±0.04</td>
<td>0.39±0.012</td>
</tr>
<tr>
<td>El-Serw</td>
<td>120.2±0.70</td>
<td>20.3±0.10</td>
<td>19.3±0.15</td>
<td>9.3±0.19</td>
<td>8.75±0.08</td>
<td>0.35±0.02</td>
</tr>
<tr>
<td>Faraskour</td>
<td>116.5±0.14</td>
<td>19.2±0.21</td>
<td>19.4±0.13</td>
<td>8.5±0.23</td>
<td>8.2±0.04</td>
<td>0.33±0.01</td>
</tr>
</tbody>
</table>

Table 3: Summary statistic of sizes of heterophyid metacercariae and intensity of helminth infection in liver of hybrid Oreochromis sp

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single infection</td>
<td>Heterophyid metacercaria</td>
<td>Diameter μm</td>
<td>48.7±4.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO./mm²</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>Heterophyid metacercaria</td>
<td>Diameter μm</td>
<td>82.6±11.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO./mm²</td>
<td>3.3±0.12</td>
</tr>
<tr>
<td></td>
<td>Nematode larvae</td>
<td>NO./larvae/mm²</td>
<td>0.9±0.04</td>
</tr>
</tbody>
</table>

Histopathological Studies: Helminthes infection were restricted to the liver of 60% of the examined fish (with no parasites in the other examined tissues). Parasites were mainly heterophyid metacercariae and undifferentiated nematode larvae. The infected fish showed mixed infection in 33% of cases while 67% were infected only with metacercariae (single infection). Metacercariae were rounded, yellowish-brown with black pigmentation and different in sizes that related to different developmental stages (Fig. 8, 25, 28and 30).

Mean diameters of metacercariae recovered from single- and mixed-infected fish were summarized in Table 3. Diameters of metacercariae of mixed-infected fish were significantly higher as compared to those of single infection ($P < 0.05$).

Table 4: Liver of collected Oreochromis niloticus fish from the nine studied stations showed many histopathological changes

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Site</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty degeneration</td>
<td>1, 8</td>
<td>2, 3, 36</td>
</tr>
<tr>
<td>Degeneration in hepatocyte</td>
<td>1, 3, 5, 6, 7, 9</td>
<td>5, 11, 23, 24, 27, 30, 31, 40</td>
</tr>
<tr>
<td>Necrosis in hepatocyte</td>
<td>1, 3, 5, 7</td>
<td>6, 12, 23, 33, 34</td>
</tr>
<tr>
<td>Congestion in blood vessel</td>
<td>3, 5, 6</td>
<td>7, 15, 20, 22, 28, 29</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>3, 6, 7, 5</td>
<td>8, 14, 26, 30, 24</td>
</tr>
<tr>
<td>Parastatic forms</td>
<td>2, 7, 8, 9</td>
<td>9, 10, 32, 37, 38, 39, 40</td>
</tr>
<tr>
<td>Edema</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Hemosidrin</td>
<td>2, 6, 7, 8</td>
<td>13, 15, 25, 34, 35</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>4, 5</td>
<td>16, 17, 19</td>
</tr>
<tr>
<td>Branching</td>
<td>5</td>
<td>21</td>
</tr>
</tbody>
</table>

Intensity of metacercariae per mm² of liver tissue was higher in case of mixed infection ($P < 0.01$) whereas, more than 60% of fish had more than three metacercariae.
Fig. 2-16: Histological sections in liver of *O.niloticus* fish stained with H&E. Fig. 1) showing normal (x400), figs.3-5) showing fatty degeneration (x400) and 6) showing degeneration (x100) colecting from site 1, figs.7&8) showing parasite cyste colecting from site 2(x400),figs.9-12) showing congestion,hemorrhages,degeneration and necrosis colecting from site 3(x400), figs.13-16) showing hemosidren, hemorrhages and congestioncolecting from site 4(x400)
Fig. 17-28: Histological sections in liver of O.niloticus fish stained with H&E. (17-23) showing hemorrhages, congestion, branching and degeneration (x400) colecting from site 5, 24-28) showing degeneration, hemorrhages and hehosidren (x400) colecting from site 6.
Fig. 29-40: Histological sections in liver of *O. niloticus* fish stained with H&E. Figs. 29-34) showing congestion, hemorrhages, degeneration, parasite, necrosis and hemosidren (x400) colecting from site 7, figs. 35-37) showing hemosidren, fibroses, parasit (x400&100) colecting from site 8, figs. 38-40) showing parasite cyste (x400) colecting from site 9.
per one mm². The intensity of infection with undifferentiated nematode larvae was 0.9 ± 0.04 spec. per mm³ of the liver (Table 3).

Histological examination of mixed-infected liver showed invasion of undifferentiated nematode larvae and heterophyid metacercariae to liver parenchyma with a notable degeneration to the surrounding area.

The histopathological response to infection was symbolized by a cytoplasmic vacuolation, fatty degeneration and lymphocytic infiltration and hepatocellular focal necrosis (Figs. 30, 32–40). In addition to the above, a noticeable damage to pancreatic cells was found (Fig. 12). In a single-infected fish, the severity of histopathological changes in liver was limited as compared to mixed infection (Figs.8, 9). However, in all cases, there was no inflammatory response to the parasites observed in the tissues. A high depletion of polysaccharides material was observed in the hepatocytes of mixed infected fish as compared to single one (Figs. 7, 8).

Histological examination of the liver samples of the studied fish species showed fatty degeneration, degeneration and necrosis in hepatic cells. Also showed congestion, hemolysis, parasitic forms, edema, hemosidrin, hemorrhage and branching in blood vessels. These occur by severe degree in fish obtained from sites 3& 4 that received industrial wastes and sites 2& 5 that received sewage wastes.

DISCUSSION

Bioaccumulation of heavy metals does not only depend on the structure of the organ, but also on the interaction between metals and the target organs [17]. Mersch et al. [18] stated that fish could accumulate trace metals and act as indicators of pollution.

Iron is an abundant and important element, unsurpassed by any other heavy metals in the earth's crust [19]. The increase of iron accumulation in fish liver in this study may be related to the increase of total dissolved iron in Nile water and consequently increase the free metal iron concentration and thereby lead to an increase in metal uptake by different organs [8,20]. Haggag et al. [21] and Yacoub [22] observed accumulation of iron ligand protein (Hemosidrin) scattered in liver section of fish exposed to high iron concentration.

Copper is a fundamental micronutrient to all forms of life in enzyme activity or random rearrangement of natural protein [23]. The elevation of copper accumulation in this study may be due to industrial and sewage wastes. These results agree with those obtained by Ibrahim and Mahmoud [24] and Tayel et al. [8] who revealed that this increase is anticipated to industrial, drainage and sewage effluents. Also, it may be due to elevated metal–binding protein synthesis as recorded by Yacoub [22].

Zinc is an essential element and is a common pollutant as well. Mining smelting and sewage disposal are major source of zinc pollution. Fish take it up directly from water, especially by mucous and gills [25]. The high accumulation of zinc in studied fish liver agrees with Hamed [26]. Ibrahim and Mahmoud [24] revealed that this increase is anticipated to industrial effluents from Tulkha Electricity station and sewage from El-Rahawy drain respectively.

The relatively higher zinc concentration in the liver of the different fish species may be due to the role of zinc as an activator of numerous enzymes present in the liver as recorded by Yacoub, [22] and Cogun et al. [27].

Manganese functions as an essential constituent for bone structure, reproduction and normal functioning of the enzymes system [28]. It is toxic only when present in higher amount, but at low level is considered as micronutrient [29]. The high accumulation of Manganese in fish liver obtained from site 4 may be due to industrial effluents from Shoubra El-khema electricity station. These results agree with those obtained by Yacoub [22].

Lead is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes [30]. It is known to inhibit active transport mechanisms, involving ATP, to depress cellular oxidation-reduction reactions and to inhibit protein synthesis [31]. Lead was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs [32] and to impair the embryonic and larval development of fish species [33]. In comparison with Yacoub [22], lead concentration in fish liver of O. niloticus ranged between 3.2µg/g dry weight at Helwan and 4.4 µg/g dry weight at ElKanater El-Khyria which were lower than the lead values reported in present study 3.9 µg/g dry weight at El-Hawamdia and 10.5 µg/g dry weight at Kom Hamada. The increase of lead level is due to the discharge of industrial, sewage and agricultural wastes in the investigated area. The high level of lead may be attributed to high lead concentration in water [8].

Cadmium is highly toxic non – essential heavy metal and it dose not have a role in biological processes in living organisms. Thus even in low concentration,
cadmium could be harmful to living organisms [30]. The values of cadmium accumulation in present study (0.10 – 1.30) µg/g dry weight were higher than those obtained by Yacoub [22] as 0.04 – 0.28 µg/g dry weight. High accumulation of cadmium in liver may be due to its strong binding with cystine residues of metallothionein [8].

Liver of fish is responsible for the digestion, filtration and storage of glucose. It is found in the anterior part of the body cavity as a brownish red mass. The liver also produces many enzymes that stored in the gall bladder. These enzymes assist in the break down of food. The liver functions to store food energy [8].

The present study suggests a strong link between heavy metals and lesions in the liver. Sorensen et al. [34] cited that heavy metals in Elbe were might cause liver damage. Aly et al. [35] obtained similar results after exposure of Clarias gariepinus to lead pollution. They found that the vacuolar degeneration and necrosis of hepatocytes may appear after 3 days but after 2 weeks, hemolysis of red blood corpuscles and vacuolar degeneration as well as necrosis of hepatocytes were observed. Similar alterations in the liver of Tilapia zilli and Clarias gariepinus were observed in fishes living in Nile water polluted with heavy metals and ammonia [8, 24, 36, 37].

Yacoub and Abdel-Sater [38] studied the effect of heavy metals on some fishes inhabiting Bardawil lagoon. They observed degeneration and vacuole necrosis in hepatocytes, with, hemolysis and hemosidrin pigments.

The fatty degeneration changes in studied liver may be due to a decrease in the rate of utilization of energy reserve or pathological enhance synthesis [39]. In addition, the abnormal accumulation of fats in experimental animal could be due to induced imbalance between fat production and utilization [40]. In general, fish livers contained hemosidrin pigments that may result from destruction of erythrocytes. Breakdown of hemoglobin converting into hemosidrin was responsible for the brown deposits within hepatic tissues [41]. Several factors have been held responsible for the abnormal accumulation of hemosidrin in the liver tissue, some were named by Strassmann [42] and Mazhar et al. [43] to be rapid and continue destruction of Erythrocytes with increased hemolysis and damage of the iron metabolism. Also high amount of iron accumulation in studied fish liver leading to abnormal accumulation of hemosidrin in the same liver sample [8, 24, 44]. Some studies also revealed a link between hepatic alteration and hemosidrin pigments [45, 46].

Fish diseases and histopathology, with a broad range of causes, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure [47]. Wild fish have greater parasite diversity but with lower population abundance and the reverse is true for cultured fish [48]. Consequently, the present study revealed that only undifferentiated nematode larvae and/or heterophyid metacercariae parasitized the liver of a high percentage of examined cultured tilapia.

It could be concluded that. The areas of investigation along the River Nile are affected by industrialization, agricultural and sewage effluents, which affect water quality, fauna, fish production and human health. It is recommended to treat different wastes before discharging to the river Nile stream.

REFERENCES


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