Assessing Health Risk of Heavy Metal Ingestion for Fish Consumption in Idku Lake, Egypt

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ABSTRACT
Carcinogenic and other health risks associated with consuming fish have been evaluated using the selected six fish species including Dicentrarchus labrax, Clarias gariepinus, Sarotherodon galilaeus, Oreochromis aureus, Oreochromis niloticus and Tilapia zillii from Idku Lake, Egypt. To estimate the non-carcinogenic (estimated daily intake (EDI), target hazard quotient (THQ) and carcinogenic human health risks (CR), the concentrations of heavy metals (Fe, Cr, Ni, Pb and Cd) in the liver and muscles tissue of six fish species were addressed. The estimated daily intake (EDI) showed that the highest concentration was in iron but this value was the only value in the heavy metal concentrations that didn't exceed the permissible limit. The total values of this indicator (tTHQ) were determined for fish species, and a noticeable increase was recorded in the values for the lead element. However, the values range are less than 1.00, indicating no discernible health. Clarias gariepinus had values of 1 that can affect human health in the long term. While for carcinogenic human health risks (CR), the highest value was recorded in the liver of clarias gariepinus fish, and it exceeds the recommended ratios. All the remaining values of heavy metals were within the recommended ranges, which indicates that there is no tangible health risk to humans.

INTRODUCTION

One of the main issues affecting people in Egypt is heavy metal contamination, particularly in five coastal lakes (Manzala, Idku, Burullus, Mariout and Bardawil Lakes). Heavy metal contamination has increased as a result of industrial waste, geochemical structure, agricultural practices and mining operations (Al Naggar et al., 2018). These lakes were a significant natural resource for Egypt's fish production, contributing more than 40% of the nation's total fish output, but as of now, that proportion has dropped to less than 12.22% (GARFD, 2013). The third largest wetland region in the Nile Delta is Idku Lake. This lake provides for roughly 15% of Egypt's total commercial fishing areas (Abdel-Hamid, 2017). Two major drains, El-Khairy and Barsik drains, provide the lake with enormous amounts of drainage water. El-Bousely, Idku and Damanhour sub-drains are the three drains from which El-Khairy Drain draws its water. These drains are to
blame for the lake's increased contamination and the rise in heavy metal levels that negatively impacted the fishing (Shetaia et al., 2020). A significant issue endangering the existence of aquatic organisms, including fish, is the pollution of the aquatic environment by inorganic and organic pollutants (Aly et al., 2020). These pollutants may have an impact on human life if people consume contaminated fish and other aquatic foods from this habitat (Aderinola et al., 2009). Heavy metals are thought to be a major cause of aquatic ecosystem contamination because of their potential toxicity and accumulation in aquatic ecosystems (Tscheikner-Gratl et al., 2019).

Fish increasingly dominates the human diet since it contains a lot of proteins, has low cholesterol, and has a lot of N-3 polyunsaturated fatty acids, including eicosapentaenoic acid and docosahexaenoic acid (DHA), as well as liposoluble vitamins and important minerals (Chen et al., 2006; Zalloua et al., 2007).

Due to the anthropogenic activities, these toxins are currently acknowledged as being the most dangerous for aquatic ecosystems. They can build up in fish tissues due to their non-biodegradability before entering the human diet (Akter, 2014). Thus, there has been discussion over the advantages and disadvantages of eating fish during the past few years, which has led to some confusion regarding the appropriate amount to eat or even if it is advised to do so at all (FAO/WHO). In order to assess the potential risk of fish ingestion by humans, it is crucial to identify the chemical quality of marine organisms, notably the concentration of heavy metals (Cid et al., 2001).

Fish have two primary uptake systems for metals: the gill surface (water exposure) and the digestive tract (diet exposure) (Ptashynski et al., 2002). In terms of metal toxicity in fish, the gills are one of the most important organs since they are in close contact with the water and serve as a location for metal uptake (Schlenk & Benson, 2001; Bury et al., 2003). A significant contributor to toxicity in fish is the interruption of ion transport across the gills (Schlenk & Benson, 2001). Metals are then transported via blood to other target organs, like the liver and kidney after being absorbed by the gills.

The liver is one of the most specialized tissues for metal metabolism and has a high propensity to accumulate metals (Uysal et al., 2008). It also performs crucial and intricate biological processes, such as energy metabolism, that are necessary for survival (Uysal et al., 2008). Yet, with the exception of organic mercury, fish muscle does not actively accumulate metals (Uysal et al., 2008). The brain and kidneys are two organs where metals frequently have hazardous effects. Convulsions, comas, and renal failure are just a few of the health issues that lead exposure can result in (Canl and Atl, 2003). Chronic cadmium exposure has been linked to testicular degeneration and chronic lung disease, whereas acute high doses of cadmium exposure can induce severe respiratory irritation (Canl and Atl, 2003).

Nickel (Ni) might conceivably have a comparable role to some critical metals, such as copper (Cu) and zinc (Zn) in the growth and metabolism of living organisms though there
is currently no sufficient evidence to support this (Zoroddu et al., 2019). In the case of Zn, a dietary shortage results in cutaneous issues, slowed growth and delayed sexual maturity (Kaim et al., 2013). Anemia, liver issues and vascular weakness are all symptoms of copper deficiency. Despite the fact that these metals are necessary, fish can accumulate them, and if their concentrations rise beyond the toxicity threshold, they may be hazardous to humans (Noman et al., 2022). In addition to being poisonous for aquatic organisms, other heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are also recognized to be non-essential to human health and can pose a threat to it at extremely low doses (Burger et al., 2014).

MATERIALS AND METHODS

1. Study area

The five northern lakes in Egypt are oriented from west to east as follows: Mariout, Idku, Burullus, Manzala and Bardawil (El Kafrawy et al., 2019).

Idku Lake is situated in the northwest of the Nile delta and about 36 kilometers east of Alexandria (Fig. 1). It is between the longitudes of 30° 8’30’’ and 30° 23’ E and the latitudes of 31° 10’ 30’’ and 31° N. The lake's water surface area reached 128km² in 1973 (Sheta et al., 2022) and currently, the lake's area is about 22km². The lake's depth varies from 0.1 to 1.40 meters, with the highest depths found in the center and eastern parts (El Kafrawy et al., 2019). Through Buoghaz El-Maadyah in the northern section, it receives salt water from the sea (Sheta et al., 2022). Average salinity is 2.24 ± 2.12 PSU, and the water's temperature is 23.54 ±5.84°C (Zaghloul & Hussien, 2017).

The lake produced 8070 tons of fish in total in 2019 (CAMPAS, 2019), which is equivalent to 3.9% of the fish produced in Egyptian lakes or 5.2% of the fish produced in northern lakes (Ramdani et al., 2001) and gets freshwater drainage from the Kom-Belag and Barsik drains, two of the primary drains. Approximately 592×106 m³ of drainage water from El-Bousyey, Idku, El-Khairy, and Damanhur sub-drains carrying household, agricultural, and industrial waste, as well as effluents from more than 300 fish farms, are received by Kom-Belag drain each year (Badr & Hussein, 2010; Khalil & Rifaat, 2013). While, the Barsik drain transfers 348×10⁶ m³ of drainage water, primarily agricultural, to the lake's southern basin per year (Badr & Hussein, 2010). The lagoon mostly undergoes water movement from the east and south to the north owing to these drainage waters (Khalil & Rifaat, 2013).
Fig. 1. The right image shows the Nile delta region and the left image shows the boundary of Idku Lake

2. Preparation and analysis of samples

Six different species of common fish were randomly collected at different sites in Idku Lake; namely, *Dicentrarchus labrax*, *Clarias gariepinus*, *Sarotherodon galilaeus*, *Oreochromis aureus*, *Oreochromis niloticus* and *Tilapia zillii* (Fig. 2). Fishes were caught using nets and were kept in clean polyethylene bags, and then placed in an icebox until they were transported to the laboratory for analysis. The fish were dissected using clean stainless-steel tools to obtain muscles and liver for each type of fish. Samples were dried in an oven at 80°C for 24 hours and then ground into a fine powder (Hashmi et al., 2002). A volume of 5ml of concentrated nitric acid (60%) and 5ml of hydrogen peroxide solution (30%) were added to 1 gram of fine powder for the sample to complete the digestion process. After digestion and cooling of the samples, they were filtered through ashless filter paper and supplemented with 50 mL of deionized water. The concentrations of iron, nickel, chromium, lead and cadmium were measured using an atomic absorption spectrometer.
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3. Consumption rate limits

3.1. Estimated daily/weekly intake

The estimated daily intake was calculated according to Saha et al. (2016) using the following equation:

\[
EDI = \frac{E_F \times E_D \times F_{IR} \times C_F \times C_m}{W_{AB} \times T_A} \times 10^{-3}
\]

Where, \(E_F\) and \(E_D\) are the exposure frequency (365 days/year) and the exposure duration, respectively; \(F_{IR}\) is the ingestion rate of fish; \(C_F\) is the conversion factor to convert fresh weight to dry weight (moisture content of fish fillet); \(C_m\) is the metal concentration in the fish tissue (μg/g dry weight basis); \(W_{AB}\) is the average body weight for adults, and \(T_A\) is the average exposure time for non-carcinogens (equal to \(E_F \times E_D\)).

3.2. Health risk assessment

Non-carcinogenic risk was investigated using the target hazard quotient (THQ), which is the ratio between the estimated exposure (estimated daily intake (EDI)) and the oral reference dose (RfD) (Saha et al., 2016). RfD (mg/kg bw/day) represents an estimate of the daily oral exposure of the human population that is likely to be without an appreciable
risk of deleterious effects. The RfDs were 0.004, 0.001, 1.5, and 0.02 (mg/kg bw/day) for Pb, Cd, Cr, and Ni, respectively (Saha et al., 2016). The following equation was used to calculate the THQ:

\[ \text{THQ} = \frac{EDI}{RfD} \]

If the values of THQ were less than 1, then no adverse hazard is expected on the human health (Saha et al., 2016). While, if the values of THQ are equal to or more than 1, then the human health is affected by noncarcinogenic health risk, and the probability should increase as the THQ value increases (Saha et al., 2016). In calculating the THQ, the effect of cooking on the concentration of contaminants was not considered, and the ingestion dose was assumed to be equal to the absorbed dose of the contaminant (Saha et al., 2016).

### 3.3. Cancer risk

The cancer risk (CR) over a lifetime of exposure to heavy metals was determined. This index was estimated using the cancer slope factor according to the following equation of Peng et al. (2016) and Shaheen et al. (2016):

\[ CR = \frac{EF \times ED \times \text{CF} \times \text{CM} \times \text{CSF}}{W_{AB} \times T_A} \times 10^{-3} \]

Where, CSF is the cancer slope factor (mg/kg/day), while the other parameters were previously defined.

### RESULTS

#### 1. Elements distribution

The concentrations of heavy metals (Fe, Cr, Ni, Pb, and Cd) were measured in the muscles and livers of six fish species collected from Idku Lake (Table 1). Iron showed the highest concentration in all fish species compared to the rest of the elements and its concentrations ranged between 4.18-5.35 µg/g dry weight and 8.95-19.57 µg/g dry weight in the muscles and liver, respectively. The highest values were recorded in *Clarias gariepinus*, while the lowest values were in *Sarotherodon galilaeus*, as shown in Table 1. These results are extremely lower than those published in 2008, stating that the concentrations of iron in the muscles and liver of the Nile tilapia were 75.5 and 720.48 µg/g, respectively in Idku Lake (Saeed & Shaker, 2008). It is worth noting that, the recorded values of iron in all fish did not exceed the internationally permitted levels (43 micrograms / gram) given by the FAO/WHO.
The values of chromium ranged between 0.398-0.678 and 0.498-0.892 micrograms/gram dry weight in the muscles and liver, respectively, in the fish species. The highest values were recorded in *Clarias gariepinus*, while the lowest values were in the Nile tilapia. These findings are matching with the previous results in Nigeria (Omozokpia *et al.*, 2015). It was noticed that the recorded values of chromium in all fish did not exceed the internationally permitted levels (2.3 micrograms/gram) given by the FAO/WHO.

The values of the nickel element changed within narrow limits between the different fish species. These values in the liver of the fish species were always higher than in the muscles, and the values ranged between (0.58 - 0.78 μg/gram dry weight) and (0.88 – 1.16 μg/gram dry weight) in the muscles and liver, respectively. The highest values of nickel were recorded in *Clarias gariepinus*, while the lowest value was in *Dicentrarchus labrax*, as shown in Table (1). These results are slightly higher than the previous results in Nigeria (Omozokpia *et al.*, 2015). It was denoted that the recorded values of nickel in all fish exceeded by a large percentage the internationally permissible levels (0.03 micrograms/g) given by the FAO/WHO.

The lead concentrations ranged between 1.68-2.32 μg/g dry weight and 1.95-2.57 μg/g dry weight in the muscles and liver, respectively. The highest values were recorded in *Clarias gariepinus*, while the lowest values were in *Dicentrarchus labrax*, as shown in Table (1). These findings were similar with those published before in the muscles and liver of *Oreochromis niloticus* in Idku Lake (Saeed and Shaker, 2008) but, these results were higher compared to the values recorded in *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii* in Idku Lake (Saeed, 2013). It is worth noting that, the recorded values of lead in all fish exceeded four times the internationally permissible levels (0.5 micrograms/gram) given by the FAO/WHO.

The concentrations of cadmium ranged between 0.096-0.109 and 0.095,151 micrograms/gram dry weight in the muscles and liver respectively, and the highest values were recorded in *Clarias gariepinus*, while the lowest values were in *Dicentrarchus labrax* as shown in Table (1). These results were much lower than those published in the muscles and liver of *Oreochromis niloticus* in Idku Lake (Saeed & Shaker, 2008). It is also considered lower compared to the values recorded in *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii* in Idku Lake (Saeed, 2013). It is clear that the recorded values of cadmium in all fish exceeded more than twice the internationally permitted levels (0.05 micrograms/gram) given by the FAO/WHO.
Table 1. Concentrations of some heavy metals in the muscles and liver of some different fish species from Lake Idku (micrograms / mg dry weight)

<table>
<thead>
<tr>
<th>Fish</th>
<th>Fe</th>
<th>Cr</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
<td>Liver</td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Dicentrarchus labrax</td>
<td>7.84</td>
<td>5.63</td>
<td>0.635</td>
<td>0.53</td>
<td>0.78</td>
</tr>
<tr>
<td>Clarias gariepinus</td>
<td>19.57</td>
<td>8.95</td>
<td>0.892</td>
<td>0.678</td>
<td>1.16</td>
</tr>
<tr>
<td>Sarotherodon galilaeus</td>
<td>5.39</td>
<td>4.18</td>
<td>0.512</td>
<td>0.492</td>
<td>0.81</td>
</tr>
<tr>
<td>Oreochromis aureus</td>
<td>5.93</td>
<td>4.53</td>
<td>0.632</td>
<td>0.487</td>
<td>0.86</td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td>7.42</td>
<td>4.23</td>
<td>0.498</td>
<td>0.398</td>
<td>0.84</td>
</tr>
<tr>
<td>Tilapia zillii</td>
<td>9.17</td>
<td>4.48</td>
<td>0.689</td>
<td>0.458</td>
<td>0.93</td>
</tr>
<tr>
<td>PL</td>
<td>43</td>
<td>2.3</td>
<td>0.03</td>
<td>0.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

2. Human health risk

2.1. Non-carcinogenic health risk

2.1.1 The estimated daily intake (EDI). The daily consumption index was calculated according to the concentrations of each metal in the fish samples that were collected. The average values for this indicator (EDI) showed a descending order of iron > lead > nickel > chromium > cadmium. The EDI values in liver were higher than those recorded in muscle in all fish species. Although the index values for the iron element were the highest values and ranged between 3.53-16.78 mg/kg BW/day, they did not exceed the recommended ratios (45 mg/kg BW/day). While, the values of the remaining elements exceeded the recommended ratios by a large amount, as shown in Table (2).

After comparing these results with other studies, we find that the values in this study are much higher than the values recorded in the muscles of some Oreochromis niloticus in Fosu Lake in Ghana (Akoto et al., 2014). On the other hand, these values are consistent with the values of Caridea recorded in the Selangor region of Malaysia (Rajan & Ishak, 2017).
2.1.2 The target hazard quotient (THQ). The target hazard quotient (THQ) is an indicator used to estimate potential health risks associated with long-term exposure to various chemical pollutants, especially heavy metals. The target hazard quotients (THQs) for the heavy metals were estimated for the selected fish species in Idku Lake, as shown in Table (3).

It was noted that, the values of the indicator were higher in the liver compared to their values in the muscles of fish. *Dicentrarchus labrax* had the lowest values for this index, while *Clarias gariepinus* had the highest values. A clear increase was recorded in the values of (THQ) for the element of lead, and it ranged between 0.360 & 0.551, while the values (THQ) for the iron element ranged between 0.007 & 0.024.

The total values of this index (tTHQ) were calculated for all fish species. Generally, the values ranged between 0.505 & 0.806, which indicates that there is no tangible health risk to humans but values close to 1 such as in *Clarias gariepinus* remains more dangerous in the long-term effect on the human health. These results are similar with the values recorded in the province of Selangor in Malaysia (Rajan & Ishak, 2017).

### Table 3. The target hazard quotients and the total values of this indicator for heavy metals studied in different fish species from Idku lake

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Fe Liver</th>
<th>Cr Liver</th>
<th>Ni Liver</th>
<th>Pb Liver</th>
<th>Cd Liver</th>
<th>tTHQ Liver</th>
<th>Fe Muscle</th>
<th>Cr Muscle</th>
<th>Ni Muscle</th>
<th>Pb Muscle</th>
<th>Cd Muscle</th>
<th>tTHQ Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>0.01</td>
<td>0.036</td>
<td>0.03</td>
<td>0.033</td>
<td>0.418</td>
<td>0.3</td>
<td>0.081</td>
<td>0.083</td>
<td>0.579</td>
<td>0.505</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clarias gariepinus</em></td>
<td>0.024</td>
<td>0.051</td>
<td>0.058</td>
<td>0.038</td>
<td>0.591</td>
<td>0.412</td>
<td>0.129</td>
<td>0.093</td>
<td>0.806</td>
<td>0.592</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sarotherodon galilaeus</em></td>
<td>0.007</td>
<td>0.029</td>
<td>0.028</td>
<td>0.035</td>
<td>0.433</td>
<td>0.429</td>
<td>0.105</td>
<td>0.089</td>
<td>0.609</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oreochromis aureus</em></td>
<td>0.007</td>
<td>0.036</td>
<td>0.037</td>
<td>0.03</td>
<td>0.461</td>
<td>0.454</td>
<td>0.103</td>
<td>0.089</td>
<td>0.644</td>
<td>0.606</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>0.009</td>
<td>0.028</td>
<td>0.036</td>
<td>0.031</td>
<td>0.45</td>
<td>0.386</td>
<td>0.98</td>
<td>0.082</td>
<td>0.621</td>
<td>0.527</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tilapia zillii</em></td>
<td>0.011</td>
<td>0.039</td>
<td>0.026</td>
<td>0.04</td>
<td>0.491</td>
<td>0.429</td>
<td>0.105</td>
<td>0.09</td>
<td>0.687</td>
<td>0.583</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2. Carcinogenic health risk

2.2.1. The carcinogenesis risk index (CR). The carcinogenesis risk index is an indicator used to estimate the risk of cancer when exposed to long-term chemical pollutants, especially heavy metals based on the cancer regression coefficient (CSF) for each substance or chemical element. The index (CR) of the heavy metals were calculated for all elements, except iron since it has no coefficient (CSF). Generally, there is a significant increase in the values of the index (CR) in the liver, compared to those in the muscles of fish species and the values ranged between $(1.1 \times 10^{-3} - 4.1 \times 10^{-5})$ (Table 4).

The highest value was recorded in *clarias gariepinus* fish, exceeding the recommended limit ($10^{-4}$). This means that the human health can be affected in the long-term. While, the values in the remaining species were within the recommended range and indicated that there is no tangible health risk to humans. This study showed the similarity with the values for *Labeo pseudocoubie* in Nigeria (Uche et al., 2017).

**Table 4.** The values of the carcinogenesis risk index of heavy metals studied in different fish species from Idku lake

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Fe</th>
<th>Cr</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
<td>Liver</td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.00027</td>
<td>0.00023</td>
<td>0.00033</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.00038</td>
<td>0.00029</td>
<td>0.0005</td>
</tr>
<tr>
<td><em>Sarotherodon galilaeus</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.00022</td>
<td>0.00021</td>
<td>0.00035</td>
</tr>
<tr>
<td><em>Oreochromis aureus</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.00027</td>
<td>0.00021</td>
<td>0.00037</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.00021</td>
<td>0.00017</td>
<td>0.00036</td>
</tr>
<tr>
<td><em>Tilapia zillii</em></td>
<td>Absent</td>
<td>Absent</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

**CONCLUSION AND RECOMMENDATION**

In this study, the concentrations of heavy metals (Fe, Cr, Ni, Pb, and Cd) were studied for six fish species from Idku lake. The results showed that the concentrations of heavy metals in all selected fish samples were within the permissible levels, except for the concentrations of nickel, lead and chromium because of the increased anthropogenic activities and the drains along the lake.

The human health risk indices such as the estimated daily intake (EDI), the target hazard quotient (THQ) and the carcinogenic risk index (CR) were estimated in this study to identify if there is any potential health hazard for consumers or not. The results of EDI for all elements were exceeded the recommended ratios except for iron. While THQ indicated that there is no tangible health risk to humans, but values close to 1 such as in *Clarias gariepinus* remains more dangerous in the long-term effect on the human health.
The results of CR revealed that the highest values were recorded in the liver of *Clarias garipinus*, which exceeds the recommended health percentages. While, the values in the remaining species were within the recommended range and indicated that there is no tangible health risk to humans.

From this study, it was illustrated that there is a potential human health risk for some heavy metals in Idku lake, especially the concentrations of nickel, lead and chromium in all fish species. These high concentrations are due to the drains and fish farms around the lake. As a result, the water quality of the lake under study was negatively affected.

Thus, the following recommendations are proposed to enhance the environmental status of Idku Lake:

1- The drainage water of the eastern and the western drains in Idku lake must be treated to reduce the pollution levels.

2- Fish farms and human activities surrounding the lake must be decreased because these are the main causes that deteriorate the water quality along the Idku lake.

3- Purification and deepening of Buoghaz El-Maadyah to allow entering the large quantities of the salty water as the only outlet for sea water to the lake.

4- Treating sewage that were discharged into the eastern and southern parts of the lake to improve water quality.

**REFERENCES**


