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Some Heavy Metals Concentration in Water, Muscles and Gills of *Tilapia Niloticus* as Biological Indicator of Manzala Lake Pollution

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Abstract

Environmental pollution is a worldwide problem, heavy metals belonging to the most important pollutants. The progress of industries has lead to increased emission of pollutants into ecosystems. Manzala Lake is one of the most important aqua systems, which receives effluents discharges from heavily industrialized and highly populated settlements. Metals tend to accumulate in water and move up through the food chain. So, studies to ascertain the level of heavy metals in environment and determine potentially hazardous levels for human are necessary. In this study heavy metal (Pb, Cd, Fe, Cu, and Zn) were determined in different tissues (muscle, gill) of Tilapia nilotica from the south part of Manzala lake (Egypt) to assess the lake water pollution with those toxic metals. The range of Fe was (0.16-0.54 µg/L) in water from sampling sites, with no significant difference between the three sites, while, the level was (0.066-1.13 µg/g) in muscle and fish gills. The level of Zn was (0.08-0.26 µg/g) in muscle and fish gills, but long-term disposal of wastewater into the lake resulted in high level of Zn and Cu in the tissues and gills of the fish. The concentration of lead was (0.05-0.31 µg/L) in water with the highest level in site 3, the discharge point of industrial effluents and (0.09-0.46 ug/g) in muscle and fish gills. The levels of Pb in this study were higher than the maximum permissible limits (FAO, UNEP, FEPA, WHO and ECR. no 2360/ 2007) for human consumption. In conclusion, the levels of heavy metals observed in the fish and water samples can be considered as a serious matter. More safe and economic methods for the elimination of heavy metals from contaminated waters are needed and continuous assessment of the level of pollution of the lake waters and fish with heavy metals is also necessary. Safe disposals of domestic sewage and industrial effluents as well as enforcement of laws enacted to protect our environment are therefore advocated.

Keywords: Heavy Metal; Tissue; Gill; Manzala lake; Tilapia nilotica; Pollution

Introduction

Environmental pollution is a worldwide problem, heavy metals belonging to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. Manzala Lake is one of the most important aqua systems, which receives disposal of industrial chemicals from many drains (such as Bahr El-Bakar) and agricultural pollutions (from Bahr Hadose). In addition, it was contaminated by Cairo and Delta sewage drainage system [1]. Metals tend to accumulate in water and move up through the food chain. So, studies to ascertain the level of heavy metals in environment and determine potentially hazardous levels for human are necessary. It is well known that this metal easily accumulates in fish tissues such as bones, gills, kidneys, liver, and scales [2]. Heavy metal ions do not degrade into harmless end products and will be toxic to many life forms [3]. Due to their toxicity and accumulation in biota, determination the levels of heavy metals in commercial fish species have received considerable attention in different countries [4]. There has been an increasing interest in the utilization of fishes as bio-indicators of the integrity of aquatic environmental systems in recent years [5]. Fish lie at the top of the aquatic food chain and may concentrate large amounts of some metals from the water. Heavy metals enter the body of fish through the skin or gills via the dissolved phase and through the digestive tract via the food [6-8]. Because waters generally have low heavy metals levels, even when high concentrations are found on the bottom, food is a significant source of these elements for fish [9,10]. The concentration of heavy metal were found to be higher in the liver, kidneys and gills than in the gonad and muscle tissues in some fish species [12,13] and the concentrations in the tissue of freshwater fish vary considerably among different studies. Therefore, the aim of the present study was to investigate the bioaccumulation levels of the heavy metals: Lead (Pb), Cadmium (Cd), Iron (Fe), Copper (Cu), and Zinc (Zn), and their accumulation in the muscle tissues and gills of the commercially important and common, eatable fishes (*Tilapia niloticus*) that are found in Manzala lake.

Materials and Methods

Description of site

Lake Manzala is the largest of the four brackish coastal lakes fringing the Nile Delta. The lake lies between 31' 45, 32' 15 E and 31' 00, 31' 35 N. It is bordered by Suez Canal from east, Damietta branch of Nile from west and Mediterranean Sea from north Figure 1. The lake connected to the Mediterranean Sea via fiver outlets, permitting exchange the water and biota between the lake and the sea. These outlets are (EL-soufara, El-Boughdady) at Damietta, El-Gamil , new El-Gamil , and Cannal EL-Etisaal) at Port Said. The study area lie at the southern part of the lake which far from the sea and the coastal area, so the sea water cannot reach it and make circulation of the water between these of the lake and the sea to remove pollution.

Sample collection

1- Water samples were collected from a depth 40 cm from three

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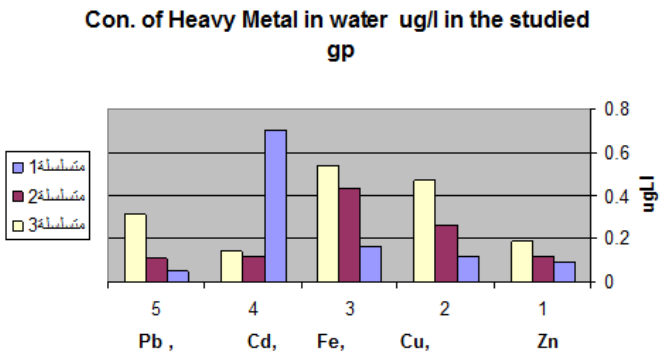


Figure 1: Concentration of heavy metal water of the studied GPS.

Site / Prameter	Pb	Cd	Fe	Cu	Zn
Site: 1	0.05	0.07	0.16	0.12	0.091
Site: 2	0.11	0.12	0.34	0.26	0.12
Site: 3	0.31	0.14	0.54	0.47	0.19

Site 1: AL-atwey Damietta, Site 2: AL-Gmalia Dakahlyi and Site 3: Materyia Dakahlia.

Table 1: Concentration of heavy metal in µg/L in water in the studied group.

sites (one sample from each) using automated water sampler (21 cc capacity) and stored in amber-colored polyethylene bottles (1 L) pre-washed with 1 (N) HNO_3 and de ionized water. To prevent further oxidation or any fungal growth 5mL concentrated HNO_3 was added to the sampled water.

2- Forty five (45) adult fish sample fifteen (15) each of *Tilapia nilotica* was procured from fishermen around the lake from three different sites. AL-Atwey Damietta site (1), AL-Gamalia Dakahlia site (2) and Materyia Dakahlia sites (3). These fish species were put in sterile polythene bags and taken in icebox to the laboratory where they were washed with running tap water to remove dirt. All the fish samples were then separately stored inside deep freezer at about -10°C and were allowed to thaw, scales were removed and washed with running water before dissected with sterile scissors to remove gills, operculum, vertebrae, heart, muscles and kidney. These were transferred into sterile sample bottles, labeled and kept for digestion and analysis of heavy metals.

Samples treatment and analysis

1- Fish muscle tissues and gills were taken from each specimen after removing the scales and skin, and each was cut into small pieces, ground well thoroughly to achieve homogeneity and used to prepare the ash solution using Inductively Coupled Plasma Emission Spectroscopy (plasma 400 emssion spectrophotometer, perkin Elmer, Norwalk Connecticut, USA) ICP-ES analysis. About 4 g of the each sample were kept in muffle furnace on a hot plate at 550°C for 3 hours to obtain the ash. Ash was dissolved in 10 ml of 20% HCl then filtered in a 100 ml volumetric flask and the volume was completed with distilled water to 50ml. Each ash solution was analyzed for heavy metals), Lead (Pb), Cadmium (Cd), Iron and (Fe), Copper (Cu), and Zinc (Zn) using inductively coupled plasma- optical emission spectrometry ICP-OES.

2- Water samples were not subjected to any further treatment and were also analyzed for the same heavy metals using Inductively Coupled Plasma Emission Spectroscopy(plasma 400 emssion spectrophotometer, perkin Elmer, Norwalk Connecticut, USA) ICP-ES. The concentration of each metal was detected in a triplicate ash samples of fish muscles and gills as well as water samples, and recorded in ppm (µg/g, µg/L) respectively according to APHA [13].

Statistical analysis

Results are presented as means M ± SE, where n equals the number of fish samples from which tissues and gill were isolated. Results from all the specimens were compared using ANOVA and $P<0.05$ was considered to indicate statistical significance. Means of significant differences were separated using Duncan's Multiple Range Test.

Results and Discussion

Concentration of heavy metals in water samples

The water samples at each sampling sites were also analyzed by using ICP-ES. The concentration of Pb and Fe was very high in all sampling sites Table 1. The range of Fe was (0.16-0.54 µg/l) in water sample with no significant difference between the three sampling sites,

These results were agreed with that reported by Abd elhamid and El-Zareef [14] they gave mean value ranged from (0.10–0.43) mg/L in El-Manzala lake's water. And disagreed with that reported by Mohammed [15] who gave average values for Fe range (0.35–1.33) mg/l in water collected from different locations of Egypt. The concentration of Cd was a pit higher while the range of Pb was (0.05–0.31 µg/l) in water with increased concentration in site 3, the discharge point of the treated waste-water at Baher EL-Baker drain. The high levels of Cd and Pb in water can be attributed to industrial and agricultural discharge [16]. The high level of Pb in water of Lake Manzala and Borollus could be attributed to the industrial and agricultural discharge as well as from spill of leaded petrol from fishing boats and dust which holds a huge amount of lead from the combustion of petrol in automobile cars [17]. The high level of Pb in water of northern delta Lakes can be attributed to heavily traveled roads that run along the Lakes. Higher levels of Pb often occur in water bodies near highways and large cities due to high gasoline combustion [18].

The concentration Zn and Cu was very low and below the detection limit in all sampling sites. However, Zyadah [19] reported higher zinc concentrations in water samples ranged from (9.47-12.3 ppm) and (0.94-2.32 ppm) in El-Manzala lake's. While, Abdelhamid and El-Zareef [14] recorded that zinc concentrations ranged from (0.01-0.09 mg/L) in El-Manzala lake's water. Mohammed *et al.* [11] gave average values ranged from (0.096-0.12 mg/L) in Lake Qarun. The distribution patterns of Cu and Zn in the Lake water increased in hot seasons (spring and summer) which may be attributed to the release of heavy metals from sediments to the overlying water under the effect of both high temperature and fermentation process resulted from decomposition of organic matter [20]. These levels are higher than the permissible limits recommended by USEPA and the Egyptian laws [21]. This may be attributed to the huge amounts of raw sewage, agricultural and industrial wastewater discharged into the Lake [22]. On the other hand, Zn and Cu values are within the allowable limits according to USEPA.

Lead was detected in the three sites (0.054–0.44 µg/g) in mussel and gill with the highest level in sites (2,3), the discharge point of industrial and sewage-treated water effluents. The above data indicated the order of heavy metals accumulation in fish muscles and gills was Pb>Fe>Zn>Cu>Cd for samples of fish from both sites of collection, while the concentrations of heavy metals in the water collected from three sites had the order Fe>Cu>Pb>Zn>Cd . The trend of accumulation suggested deposition was maximum for lead and iron, and minimum for cadmium in the muscles and gills of fish samples Figure 2. In

water samples, the concentration of Pb and Fe was very high in Sites (2,3) where effluent from different sources are discharged, then the level came down at Site (1) which far from the point of industrial and sewage-treated water . This situation explains why the concentrations of these metals were high at Sites (2,3), and consequently, their levels in the water increase at Sites (2,3), which is released into the lake. The water condition was also very muddy caused by the effluent discharge into the water.

This result was in agreement of [23]. The concentrations of heavy metals including Iron, Zinc, Copper, Manganese, Cadmium and Lead (Fe, Zn, Cu, Mn, Cd and Pb) in water and sediments in northern Delta Lakes (Edku, Borollus and Manzala) and their accumulation in Nile tilapia (Oreochromis niloticus) organs (muscle, gills and liver) were investigated.

Water, sediments and fish organs from Lake Manzala showed greater concentrations of most of the studied metals than those from Lake Edku and Lake Borollus. Fe, Mn, Cd and Pb (in Lake Manzala) and Mn and Pb in Lake Borollus recorded levels above the international permissible limits in water. Gills and Liver of *O. niloticus* contained the highest concentration of most the detected heavy metals, while muscles appeared to be the last preferred site for the bioaccumulation

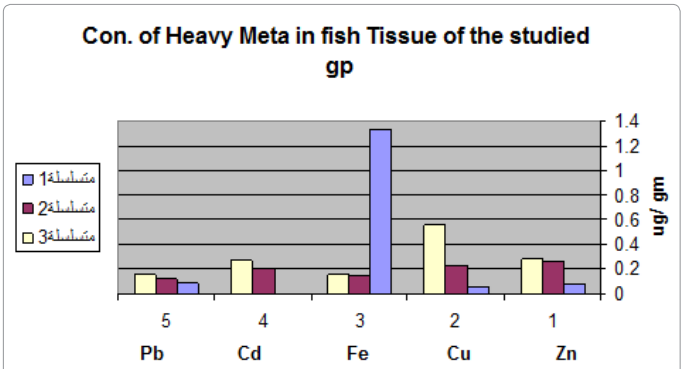


Figure 2: Concentration of heavy metal in fish tissue of the studied GPS.

of metals. The edible part of O. niloticus showed higher levels of Cd (in Lake Edku and Manzala) and Pb (in Lake Manzala). Zyadah [19] reported that the cadmium levels in El-Manzala lake's water ranged between 0.036 and 0.15 mg/L. While El-Safy and Al-Ghannam [24] found that the concentration of cadmium in the same lake's water ranged between 0.009-0.013 ppm. Moreover, El-Mezein [25] found that cadmium levels in El-Manzala lake's water took a range of 7.6-766 ppb and 10-132 ppb. On the other hand, Salbu and Steinnes [26] mentioned that Scandinavian Small Headwater lake's contained 0.004-0.54 ppb cadmium. While very low concentration were recoded in Dart's lake's water (in New York), which contained 0.7 ppb cadmium [27].

Concentrations of heavy metals in fish samples

The concentrations of heavy metals (Pb, Cd, Fe, Cu, and Zn) determined in fish muscle and gills at the three sampling sites are given in Tables 2. The highest concentration of the heavy metal in muscle and gills was recorded for Pb, followed by Fe, Zn, Cu, and Cd where Pb and Fe recorded higher concentrations in the muscles compared to the gills of fish samples collected from all sites.

Although some metals such as Zn and Cu were high in the tissues and gills of the fish, they are very low and below the detected limits in the water from where the fish samples were collected. This however is in agreement with a study in Turkey and Malaysia, where the concentration of heavy metals in fish was high even the concentration of heavy metals in the water was low [28]. El-Mezein [25] gave average value ranged from 10.82 to 19.48 and 16.2 to 18.33 mg/kg dry weight for lead in fish muscles collected from El-Manzalah lake. Ayotunde and Offen [29] found a level of (0.02 to 0.04 mg/kg) of lead in muscle tissues of some species of freshwater fish from Cross River in Nigeria and Oladimeji and Offen [30] noticed in *O. niloticus*, that the gills consistently accumulated higher amount of lead as lead nitrate. The biological effects of sub lethal concentrations of lead include delayed embryonic development, suppressed reproduction, and inhibition of growth, increased mucous formation, neurological problems, enzyme inhalation and kidney dysfunction. Because lead crosses the blood–brain barrier, it can cause various detrimental effects to the body

Site / Prameter	Pb	Cd	Fe	Cu	Zn	Weight	Length
Site: 1 Tissue	0.09 ± 0.025	0.0048 ± 0.02	1.13 ± 0.02	0.060 ± 0.02	0.08 ± 0.2	110.5 ± 0.0	14.3±0.02
Site: 2 Tissue	0.16 ± 0.02	0.27 ± 0.03	0.16	0.24 ± 0.021	0.26 ± 0.03	101.2 ± 0.03	15.8±0.02
Site: 3 Tissue	0.446 ± 0.02	0.048 ± 0.025	0.254 ± 0.02	0.112 ± 0.03	0.17 ± 0.02	123.73 ± 0.025	14.2±0.03

Site 1: AL-atwey Damietta, Site 2: AL-Gmalia Dakahlyi and Site 3: Materyia Dakahlia.

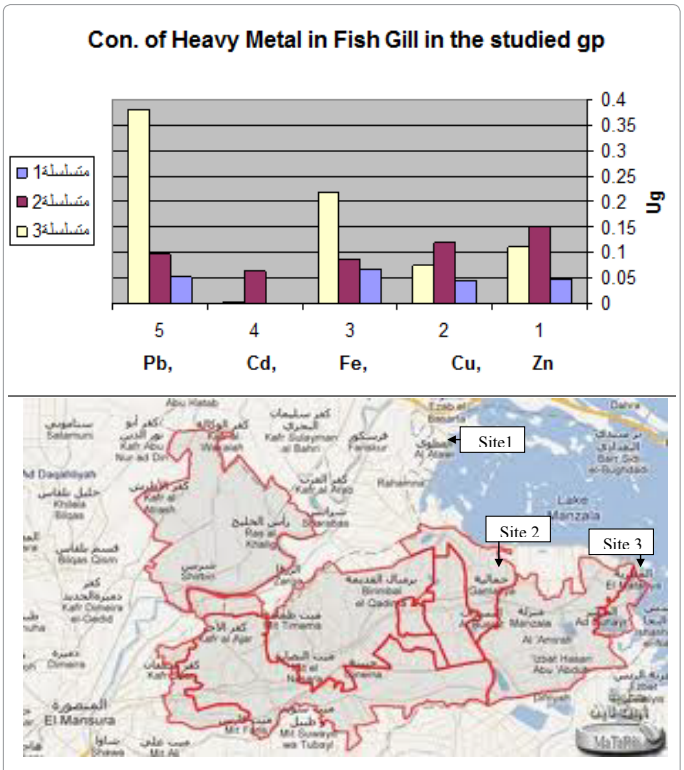
Table 2: Concentration of heavy metal in ug/kg in *Tilapia niloticus* fish (tissue) , Weight in gm and Length in cm in the studied group.

Site / Prameter	Pb	Cd	Fe	Cu	Zn	Weight	Length
Site: 1 Gill	0.055 ± 0.02a	0.00026 ± 0.01a	0.066 ± 0.2	0.044 ± 0.03	0.047	110.5 ± 0.032	14.3±0.02
Site: 2 Gill	0.096 ± 0.03b	0.065 ± 0.02	0.084	0.12 ± 0.03	0.15 ± 0.022	101.2 ± 0.03	15.8±0.02
Site: 3 Gill	0.38 ± 0.024a	0.0025 ± 0.03a	0.23 ± .0.025	0.074 ± 0.025	0.10 ± 0.021	0.033 ± 0.02	14.2±0.03

Site 1: AL-atwey Damietta, Site 2: AL-Gmalia Dakahlyi and Site 3: Materyia Dakahlia.

Table 3: Concentration of heavy metal in ug/kg in *Tilapia niloticus* fish (tissue and gill) , Weight in gm and Length in cm in the studied group.

condition, health, and life of fish [31-33]. Bioaccumulation of lead in piscine tissues is a threat to humans who may absorb considerable amounts of this metal from contaminated fish. It is common knowledge that lead adversely affects the intellectual development of children, increases arterial pressure, and contributes to cardiovascular diseases in adults (EC 2001). The level of lead in muscle and gills of *O. niloticus* was below the (0.5 µg/g) limits in Tables 3. The accumulation of cadmium was higher in muscles compared to fish gills. Dural *et al.* [34] reported highest levels of cadmium, lead, copper, zinc and iron in the liver and gills of fish species viz. *Sparus aurata*, *Dicentrachus labrax*, *Mugil cephalus* and *Scomberomorus cavalla*. Yilmaz et al. [35] reported maximum accumulations of cadmium, cobalt and copper in the liver and gills of *Leuciscus cephalus* and *Lepornis gibbosus*, while these accumulations were least in the fish muscle. However, as could be deduced from the present study, the muscles accumulate more of these metals. The levels of Pb in this study were higher than the maximum permissible limits (Food and Agriculture Organization (FAO), United Nations Environment Programme (UNEP), Federation of the European Producers of abrasives (FEPA), World Health Organization (WHO) and the Egyptian Commission Regulation (ECR). no 2360/2007) for human consumption. Cd may unlikely be harmful to human and ecological health due to the very low total concentrations [36]. Cd is toxic elements which have no known biological function and show their carcinogenic effect on aquatic biota and humans. Cadmium is widely distributed at low levels in the environment and is not an essential element for humans, animals and plants. The European and National Drinking Water Quality Standards Maximum Residue Limit (MRL) permitted in fish is 0.3 µg/g for Pb and 0.1 µg/g for Cd. The levels of lead, copper, zinc and iron in suspended particulate matter content range between 3.1–27.5 mg/kg; 0.4–11.7 mg/kg; 1–1.5 mg/kg; 1.2–1.7 g/kg, respectively. This study revealed that heavy metals



Map of Manzala lake show the three study site site.

Figure 3: Concentration of heavy metal in fish gill of the studied GPS.

pollution is mainly localized in the commercial (Rades) and fishing (La Goulette) harbours and not in the yachting (Sidi Bou Said harbour) [37]. High level of Cu was also detected in both muscle and fish gills. For the gills samples, it may be due to the fact that freshwater fish's gills might be expected to be the primary route for the uptake of water borne pollutants. WHO reported that copper toxicity in fish is taken up directly from the water via gills and stored in the liver. Copper and zinc were the most abundant metals and ranged from 11 to 63 mg/kg and from 31 to 185 mg/kg in coral tissues, respectively. The highest concentration of each metal was measured in P. furcata tissues, with copper and mercury concentrations significantly higher in P. furcata than in A. Tenuifolia at every site Figure 3. These results suggest that P. furcata has a higher affinity for metal accumulation and storage than *A. Tenuifolia* [38]. Although, the concentrations of heavy metals Fe, Cu, and Zn, determined were very low and below the detection limit, the continuous discharge from the sewage treatment into the south part of the lake might be the contributor of heavy metal accumulation and other possible pollutants in the fish samples. The high load of heavy metals is due to the presence of major sources of metal pollution, intensive human activity and discharge of municipal waste and industrial effluents map.

Our results for iron concentration in fish muscle are disagreed with Zaghloul [39] who reported that average level ranged between 10.7–30.6 and 8.6-21.3 mg/kg wet weight in muscle of

fish. Also our results were disagreed with those found by Abdelhamid and El-Zareef [14] they gave average percentage values ranged from 116.00 to 235.00 and 158.00 to 270.00 mg/kg dry weight of fish muscles from El-Manzala lake. Allowance zinc level (40–50 mg/kg) wet weight was report by WHO/FAO. Also, Zyadah [19] gave (40.84 ppm) for fish collection from El-Manzala lake.

Conclusion

In conclusion, the levels of Fe, Zn, Cu, Pb and Cd observed would have harmful effect on the health of the community in the study area. Heavy meals are one of the more serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems, thus fish species will not be safe for human consumption. Despite its extensive treatment processes for heavy metal removal from wastewater residues, heavy metals remain in the water and absorbed by fish. The concentrations of heavy metal in the tissue of freshwater fish vary considerably among different studies. Therefore, there should be a continuous assessment of pollution with metals from the mentioned sources in the lake water and other commercial lake fish, with a view to reducing the level of pollution via education and public enlightenment. Fish can be considered as an index of metal pollution in the aquatic bodies [40], Multivariate statistical techniques, such as principal component (PCA), cluster (CA), and lineal discriminant (LDA) analyses were applied for identification of the most significant variables influencing the environmental quality of water. The usefulness of the multivariate statistical techniques employed for the environmental interpretation of a limited dataset was confirmed. Alvarez and therefore, could be a useful tool to study the biological role of metals present at higher concentrations in different species of the lake fish.

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