

## Assessment of Heavy Metals Pollution and Microbial Contamination in Water, Sediments and Fish of Lake Manzala, Egypt

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**Abstract:** The objective of this study is to assess the heavy metals pollution and microbial contamination in water, sediments and fish of Lake Manzala and study the direct and indirect effect for disposal of waste water, industrial and agricultural drainage water into the lake on the environmental situation. Samples were collected from four different sites in the lake and one site in the drain. The selected sites are exposed to direct and indirect industrial, municipal waste water and agricultural drainage disposals. A site near the connection between the lake and the sea was chosen as a possible unpolluted site to compare. Three water samples and one sediment sample were collected monthly from each site for thirteen months. Fish samples (*Oreochromis niloticus*) were collected randomly for eight months from different sites of the lake. Water salinity and pH values were measured to each site. Water, sediment and fish samples were analyzed for the concentration of five major heavy metals (Mn, Cd, Zn, Pb and Cu), and two groups of bacteria (Total viable bacteria TVB - faecal coliform bacteria FCB). Results show that all the water and sediment samples were collected from five sites contain different concentration of the five tested heavy metals. The highly polluted site with Zn, Pb, Cu in sediment was found in the drain as a result of the industrial disposal. The highly polluted site with Zn and Cu in water and Cd in sediment was found in site near the industrial area as a result of direct disposal of factories nearby. Although the site near the connection between the sea and the lake was assumed to be the purest site, It was found contained a maximum concentration of Pb in water. It is possibly due to the disposal from a new natural gas factories located near this area. All the five sites were contaminated with high rate of TVB and FCB in water which is an indicator of untreated waste water which spilled directly or indirectly to the lake. The most alarming result was found when analyzing fish; all the fish samples were contaminated on surface and internally with very high amounts of TVB and FCB at gill and intestine. Fish samples also had high concentrations of analyzed heavy metals at their flesh. The mean calculated value of Pb was high up to 38 times than the allowed permissible concentration. This confirms that lake fish is highly polluted and dangerous for human health.

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**Key words:** Water, Heavy metals, Lake Manzala, Fish, Microbial contamination

### 1. Introduction

Water and soil pollution are considered to be one of the most dangerous hazards affecting not only in Egypt but also in the majority of world countries. The spoilage of water quality and water's natural balance in its environment are known as water pollution (Akman *et al.*, 2000). The aquatic ecosystem consists of several components which are directly or indirectly affected by pollution (Kosygin *et al.*, 2007). The pollution of a particular water body can always be linked to an industry, sewage or agricultural drainage (Subramanyam and Sambamurty, 2006; Sathware *et al.*, 2007). The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDG, 2002).

The most anthropogenic sources of metals are industrial, petroleum contamination and sewage disposal (Santos *et al.*, 2005; Prasanna *et al.*, 2012). Lake Manzala, the largest of Egypt's Mediterranean wetlands, and the most productive for fisheries, is suffering from land reclamation, industrial and nutrient pollution and overgrowth by water hyacinth. In the last six decades Lake Manzala subjected to various threats among of them agriculture drainage, municipal sewage and industrial wastewater. A total of 3.7 km<sup>3</sup> of fresh water (mostly from agricultural drainage) flow annually into Lake Manzala from nine major drains and canals. The most important of these are Faraskur, Al Sarw, Baghous, Abu Garida and Bahr El Baqar. Bahr El-Baqar is considered as one of the most polluted drains in Egypt (Abdel-Shafy and Aly, 2002). It receives and carries the greatest part of wastewater (3 billion m<sup>3</sup>/year) into the lake

through a very densely populated area of the Eastern Delta passing through Qalubya, Sharkia, Ismailia and Port Said Governorates. As a polluted drain flowing into Lake Manzala, Bahr El Baqar drain has received considerable concern by many scientists. Ali *et al.*, (1993) and Abdel-Azeem *et al.*, (2007) studied the effect of prolonged use of drain water for irrigation on the total heavy metals content of south Port-Said soils. Water quality, chemical composition, and hazardous effects on Lake Manzala water and living organisms caused by Bahr El-Baqar drain water has also been studied by Rashed and Holmes (1984), Khalil (1985) and Ezzat (1989). Special attention has been paid to the effect of environmental pollution from microbiological and toxicological points of view (Zaki, 1994). Lake Manzala has gradually transformed, with time, from a brackish environment (Fouad, 1926; Bishai and Yossef, 1977) to eutrophic fresh water in response to increased fresh water inputs, nutrient loading associated with agricultural land reclamation processes and due to the urban waste disposal. Many studies on pollution of Lake Manzala were done by many investigators like: Khalil (1985), Salib and Khalil (1986), Abdel Moati and Dowidar (1988) and Siegel *et al.*, (1993). Also Mekki (1996) and Ramadan and Mekki (1996) inspected to the toxicity effects by four heavy metal pollutants (Pb, Cd, Zn and Hg) in Lake Manzala on the cytogenetic components of selected crops. The metal ions can be incorporated into food chains and concentrated in aquatic organisms to a level that affects their physiological state (Abolude *et al.*, 2009). The effective of pollutants are the heavy metals which have drastic environmental impact on all organisms. Trace metals such as Zn, Cu and Mn play a biochemical role in the life processes of all aquatic plants and animals; therefore, they are essential in the aquatic environment in trace amounts. In the Egyptian irrigation system, the main source of Cu and Pb are industrial wastes as well as fungicides (for Cu), while that of Cd is the phosphatic fertilizers used in crop farms (Mason, 2002). Lake sediments are normally the final pathway of both natural and anthropogenic components produced or derived to the environment. Sediment quality is a good indicator of pollution in water column, where it tends to concentrate the heavy metals and other organic pollutants. The objective of this study is to assessment of heavy metals pollution and microbial contamination in water, sediments and fish of Lake Manzala and study the direct and indirect effect for disposal of waste water, industrial and agricultural drainage water into the lake on the environmental situation. In order to achieve that, four sites in the lake and one site in Bahr El Baqar drain was chosen to investigate.

## 2. Material and Methods

### 2.1. Site description

Lake Manzala, the largest of Egypt's Mediterranean wetlands and the most productive for fisheries, is located in the north-eastern corner of the Nile delta. Manzala is generally rectangular in shape, about 60 km long and 40 km wide, and has an average depth of 1.3 m. It is separated from the Mediterranean Sea by a sandbar, through which it is connected to the sea by three channels (*bughaz*). The salinity in the lake varies greatly; while it is low near drain and canal outflows in the south and west, it is high in the extreme north-west. Brackish conditions predominate over much of the remainder of the lake. Over 1,000 islands of varying sizes are scattered throughout the lake, some of them are inhabited. Large areas in the north-west of the lake have been turned into fish-farms, while much of the southern part of the lake has been divided into large plots and drained, in preparation for its conversion to agricultural use. A total of 3.7 km<sup>3</sup> of fresh water (mostly from agricultural drainage) flow annually into Lake Manzala from nine major drains and canals. The most important of these are Faraskur, Al Sarw, Baghous, Abu Garida and Bahr El Baqar. All of the drains discharging into Lake Manzala, the Bahr El Baqar drain is the most polluted. It carries a mixture of treated and untreated waste-water originating from Cairo and contributing much to the deteriorating water quality of the lake.

### 2.2. Samples collection

A map of Lake Manzala showing the sampling sites were presented in the Fig. (1). Water, sediments, and fish (*Oreochromis niloticus*, n = 140 fish) samples were collected from 5 different locations of Lake Manzala (Table 1) during the period from September 2008 to December 2009 (Except two months June and November 2009, in this time the Lake was closed due to security reasons). The locations were chosen so as to represent different degrees of pollution. The first site (El-Kapoty area) south of Port Said city is located near an industrial area and is exposed to industrial disposal. The second site is located near the exit of the city waste water treatment station west of Port Said city. The third site (El-Kowar) is located near the connection between the lake and Sea and also near the second exit of the waste water treatment station. The fourth site (El-Bashtir) is located at the lake near the spill of Bahr El Baqar drain to the lake. Finally, the fifth site is located inside the drain at its end before its spill into the lake. Water samples were collected monthly from a depth half meter from the water surface in 3 polyethylene bottles, acidified with nitric acid. The samples at each site were mixed in composed sample and placed in polyethylene bottle; Kept refrigerated and transferred cold to the laboratory for analysis. As well as surficial sediment samples

were collected by using core sampler according to Boyd and Tucker (1992). Then kept in cleaned plastic bags and chilled on ice box for transport to the laboratory for determination five heavy metals Zn, Cd, Mn, Pb and Cu. The parts of gills, skin, and

dorsal muscle were taken from each fish were put in small Erlenmeyer flasks and transferred in an ice box to the laboratory for microbiological and chemical analyses, which were carried out within 24 hrs from sampling.



**Figure 1: Study sites (\*) and collection samples in Lake Manzala north-eastern corner of the Nile.**

**Table (1): Location of sampling sites by global positioning systems (GPS) in Lake Manzala**

Site No.	Site name	GPS	
		N°	E°
1	El-Kapoty area	31° 14' 30.9''	32° 15' 39.2''
2	Beside Water purification plant (waste water station)	31° 15' 25.9''	32° 13' 55.3''
3	El-Kowar	31° 15' 53.1''	32° 12' 47.9''
4	El-Bashtier	31° 12' 06.6''	32° 11' 07.6''
5	Bahr El-Baqar drain	31° 11' 48.7''	32° 12' 14.9''

### 2.3. Analytical methods

#### 2.3.1. Physico-chemical analysis of water

Samples have been subjected to various analyses including water temperature at the time of sampling using a digital thermometer, depth by standard meter, current velocity (using current meter), pH value and electric conductivity (EC) by using portable devices (pH meter model HI 8314 and digital conductivity meter HI2300 Hanna Ins. Romania).

#### 2.3.2. Heavy metals in samples (water, sediment and fish)

The five heavy metals (Zn, Cd, Mn, Pb and Cu) concentrations in water were determined by the extraction method (APHA, 1998) using an atomic absorption spectrophotometer (model PYE UNICAM SP9, England). The sediment and fish samples were dried in an oven at 105 °C for about 24h, and acid digested by nitric and perchloric acid (2:1) on hotplate until the solution became clear. Then the five heavy metals described as above were determined by the same method. The concentrations of heavy metals were expressed as mg/l for water and µg/g dry wt. for sediment and fish tissues.

### 2.4 Preparation samples for microbial assay

#### 2.4.1. Water samples

Ten ml of each water samples from different sites were made serially dilutions ( $10^{-1}$  to  $10^{-5}$ ) with sterile physiological saline (0.85% wt/vol NaCl) in deionized water.

#### 2.4.2. Fish flesh

The fish body surface was wiped with 70% ethanol, and the parts of gills, skin, and dorsal muscle were taken from each fish were put in small sterilized Erlenmeyer flask. All the three parts were homogenized in a mortar aseptically. Approximately 1g of wet homogenate was put in 10 ml of sterile physiological saline. One ml of the homogenate was serially diluted ( $10^{-1}$  to  $10^{-7}$ ) according to Al-Harbi (2003).

#### 2.5. Isolation and enumeration of faecal coliform bacteria (FCB) from collected samples

The faecal coliform bacteria were enumerated by multiple tube fermentation tests as described by APHA (1998). Presumptive coliform test was carried out using MacConkey broth (Oxoid). The first set of the five tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength broth. All the tubes contained Durham tube before sterilization. The three sets of the tubes received 10ml, 1ml and 1ml of samples (water and fish) using sterile pipettes. The tubes were incubated at 44.5°C for 24-48hrs and examined for acid and gas production. Acid

production was determined by colour change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tube.

#### 2.5.1. Confirmed test

Confirmed test was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth (Oxoid) with Durham tubes. The tubes were 44.5 °C and observed for gas production. The completed test was carried out by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37 °C for 24-48hrs. Colonies developing on EMB agar were further identified as faecal coliforms (*Escherichia coli*).

#### 2.6. Enumeration of total viable bacteria from water samples

Total heterotrophic bacteria in the water samples were obtained using the pour plate method. Dilutions of  $10^{-1}$  to  $10^{-6}$  of the samples were prepared in 0.1% buffered water peptone (Oxoid) and duplicate 1ml aliquots of each dilution was inoculated into 10ml each of molten plate count agar (PCA) at 37 °C for 24 hours enumerated. Petri-dishes from dilutions containing between 30 and 300 discrete colonies were counted

and the result expressed as the numbers of bacteria per milliliter, (Anon, 1994 and APHA, 2005).

#### 2.7. Enumeration of total viable bacteria from fish samples

Bacteria were isolated from *Oreochromis niloticus* according to the technique adopted by Zaki (1994).

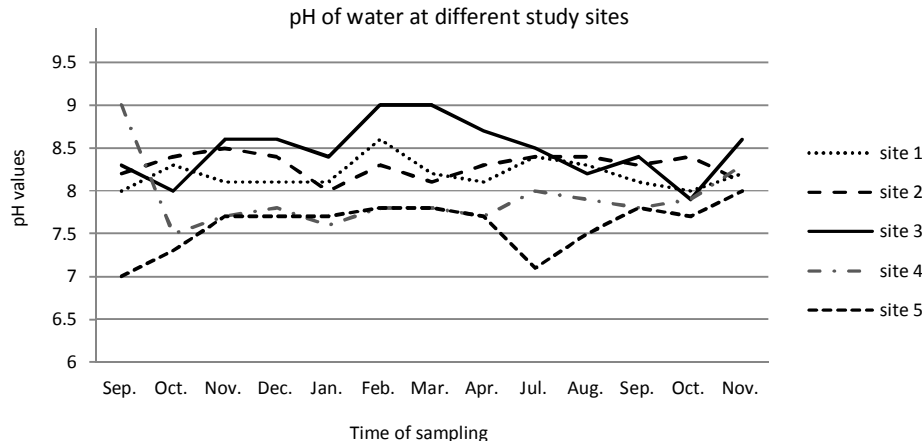
#### 2.8. Statistical analysis

One-way ANOVA and Duncan multiple range test were used to evaluate the significant difference in the concentration of different study sites. A probability at level of 0.05 or less was considered significant (Bailey, 1981). Standard errors were also estimated.

### 3. Result and Discussion

#### 3.1. Physicochemical parameters

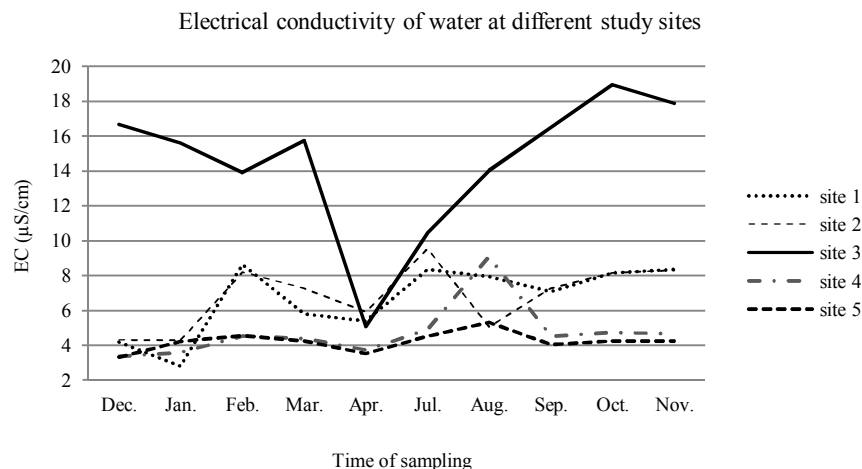
The results of pH were recorded in sites 1, 2 and 3 showing a slightly too moderately alkaline (7.9 to 9) and hence high alkalinity. It is probably due to the effect of direct industrial disposal near their location. While in site 4 and 5 the alkalinity is relatively less than the other sites. It is probably due to the nature of drain water which depend on many variables and mixture of different drainage water coming from agriculture, industry and waste water treatment stations (Fig. 2)



**Figure 2: pH values of water at different study sites of Lake Manzala from September 2008 to November 2009**

The electric conductivity (salinity) values for water samples at the study sites during the study period illustrated in Fig. (3). Water salinity is not high at the sites near or at the drain and at east of the Manzala lake. Site 3 has the higher water

salinity; it's due to location near the Sea/lake connection. However, the water salinity is up to quarter the value of sea water salinity. The measurements may give good evidence on the not effective connection between lake and Sea.



**Figure 3: The mean values of water electrical conductivity (E.C) at study sites of Lake Manzala from December 2008 to November 2009**

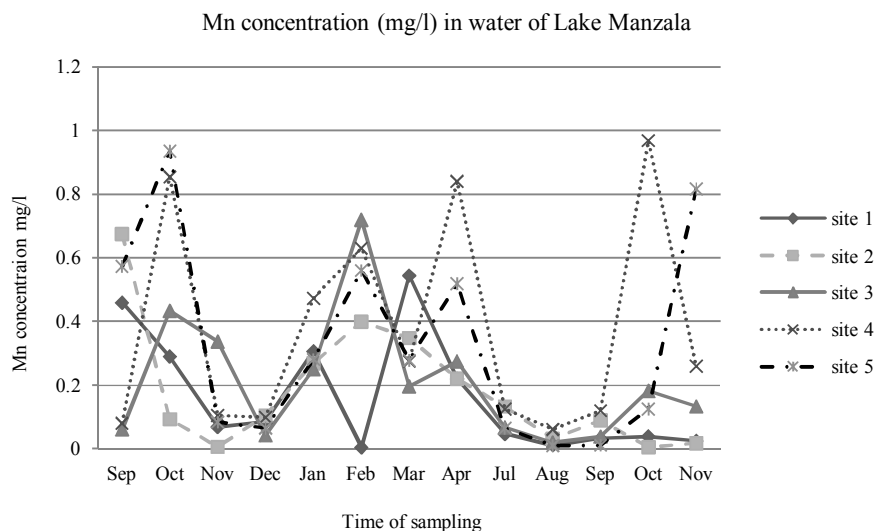
### 3.2 Heavy metals in samples (water, sediment and fish)

#### 3.2.1 Heavy metals concentrations in water and sediment

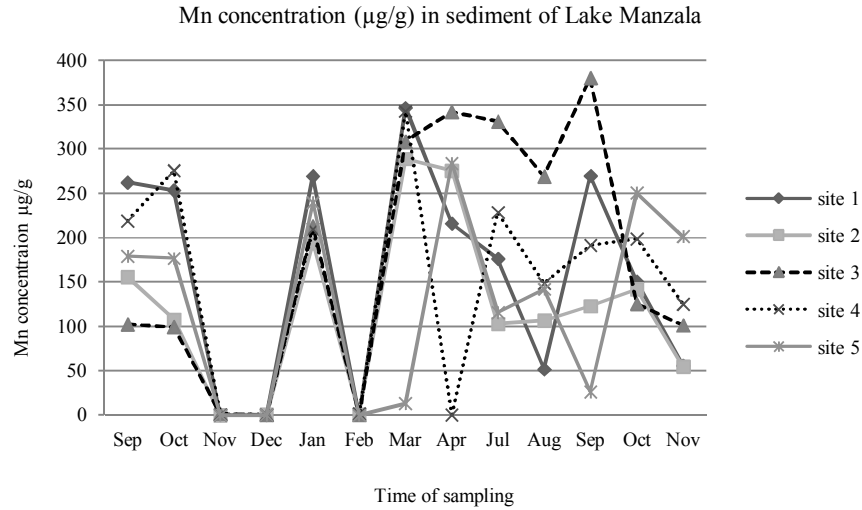
##### - Mn Concentration

The results were illustrated in Fig. (4) show that the maximum calculated amount of Mn in lake water during study period was found in site (5) Bahr El-Baqar drain (0.72 mg/l mean of 13 values) at mean pH 7.5 when water temperature mean 22 °C and water current 0.095 m/sc<sup>-1</sup>. It is probably due to the agricultural drainage water filled with fertilizers spilled into the drain. Furthermore, the fish farms adjacent to the drain use some chicken

farms residuals rich with Mn for feeding fish. On the other hand the maximum calculated amount of Mn in soil sediment was found in site (1) El-Kapoty area (mean 157.9 µg/g) at water current value 0.029 m/sc<sup>-1</sup> Fig. (5). It is probably due to the industrial disposal from the factories nearby. The very high values recorder in some sites at certain times could be attributed to some fish food residues at the sediments since the lake contains many fish catches which contain fish food provided by the owners of these fish catches. These results are in agreement with previous studies performed on Lake Manzala (Abdelhamid and El-Zareef, 1996; Abdel-baky *et al.*, 1998 and Ibrahim *et al.*, 1999).



**Figure 4: The values of Mn concentration (mg/l) in water of Lake Manzala at different study sites**

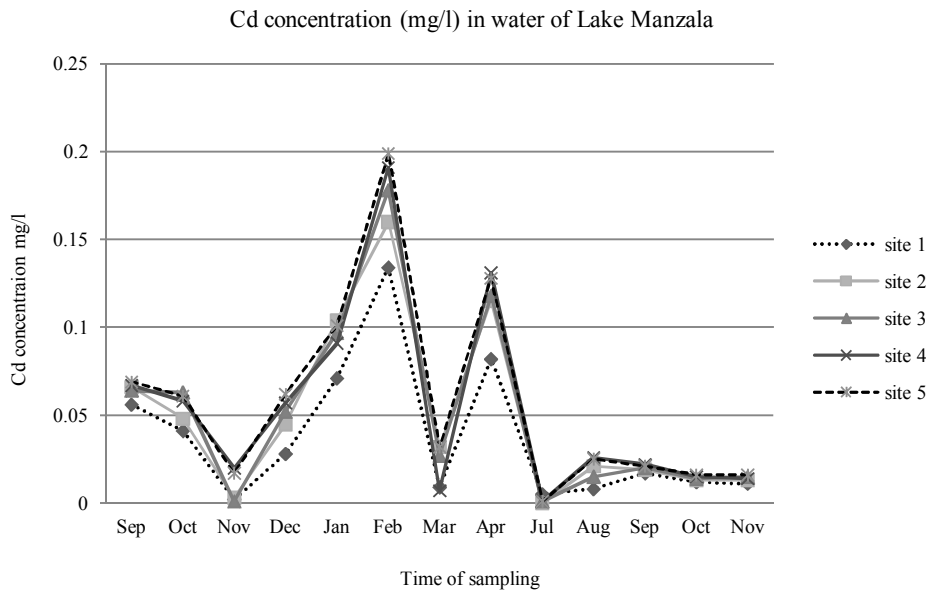


**Figure 5: The values of Mn concentration ( $\mu\text{g/g}$  dry wt.) in surfacial sediment of Lake Manzala at different study sites**

**- Cd concentration**

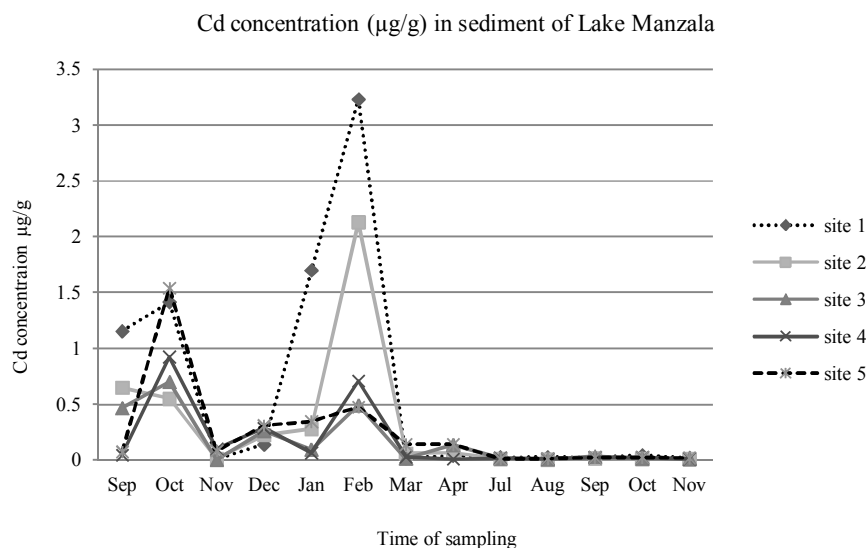
The results were illustrated in Fig. (6) show that the difference between sites in Cd concentration values is not much but the maximum calculated concentration of Cd in lake water was recorder in site 5 (Bahr El-Baqar drain, mean 0.057 mg/l) at mean pH 7.5 when water temperature mean 22 °C and water current 0.095 m/sc<sup>-1</sup> in mean. It is probably due to the industrial disposal into the drain. All the sites contain a reasonable

concentration of Cd. While the maximum calculated concentration of Cd in sediment was found in site 1 (El-Kapoty area site near the industrial area mean Cd 0.501  $\mu\text{g/g}$ ) at mean values water current 0.029 m/sc<sup>-1</sup>. It is probably due to the paint factories disposal without treatment directly to the lake Fig. (7). the previous result is in agreement with Elghobashy *et al.*, (2001) and Bahnasawy *et al.*, (2011)



**Figure 6: The values of Cd concentration (mg/l) in water of Lake Manzala at different study sites**



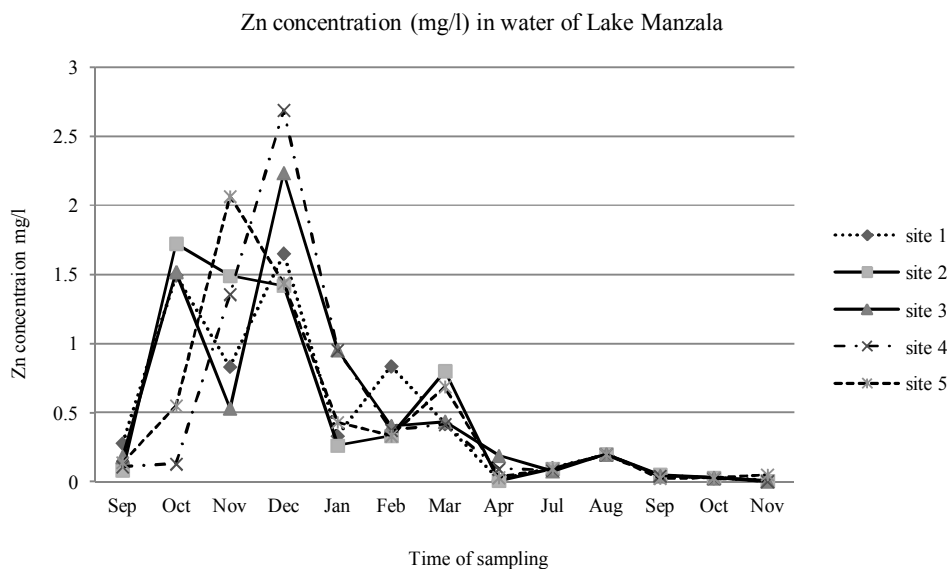


**Figure 7: The values of Cd concentration ( $\mu\text{g/g}$  dry wt.) in surfacial sediment of Lake Manzala at different study sites**

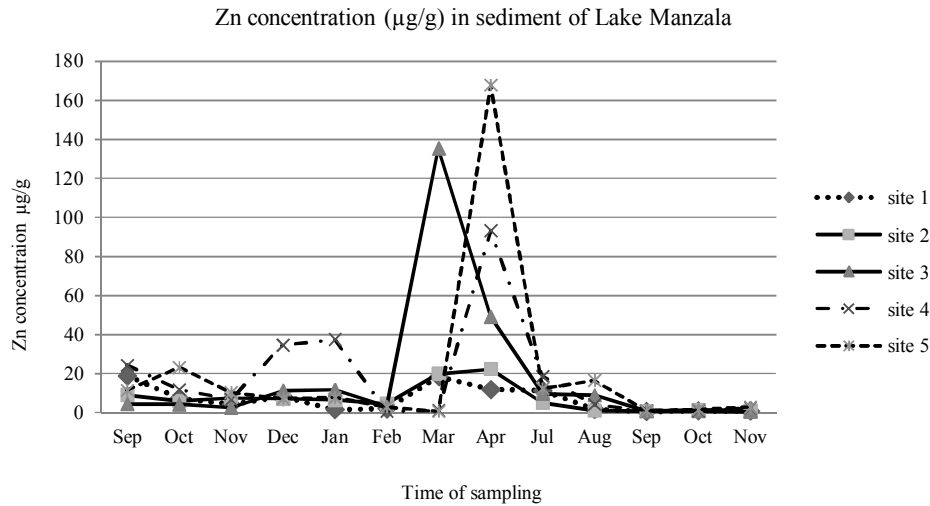
#### -Zn Concentration

The difference in Zn concentration between the five sites is not too much and all the sites expose high concentration of Zn as a result of industrial disposal spilled directly or indirectly to the lake. However, site 4 has the higher total concentration of Zn (mean  $0.589 \text{ mg/l}$ ) as a result of its exposed to the indirect industrial disposal from water coming from Bahr ElBaqar drain Fig.

(8). Also from Fig. (9), results show that the maximum calculated concentration of Zn in sediment was found in site 5 (Bahr El-Baqar drain-mean Zn  $23.5 \mu\text{g/g}$ ) when water current found at  $0.08 \text{ m/sc}^{-1}$ . It is probably due to the deposition of the industrial disposal spilled into the drain into the bottom of the drain for a long time. The same results are in agreement with Ibrahim *et al.*, (1999) and Bahnasawy *et al.*, (2011).



**Figure 8: The values of Zn concentration ( $\text{mg/l}$ ) in water of Lake Manzala at different study sites**

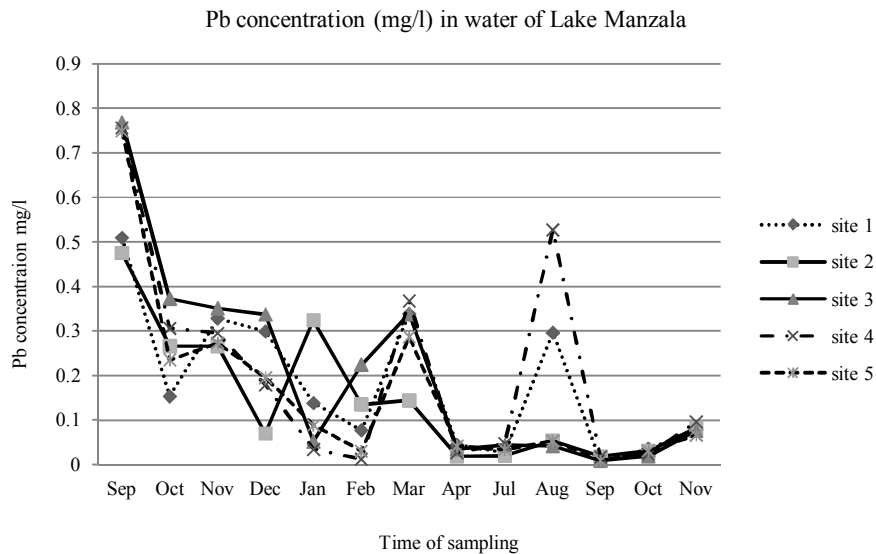


**Figure 9: The values of Zn concentration ( $\mu\text{g/g}$  dry wt.) in surfacial sediment of Lake Manzala at different study sites**

#### -Pb Concentration

Data presented in Fig. (10) showed that the Pb concentration is not high in values through all the sites but the surprising result was found in site3 [El-Kowar] near the connection between Sea and Lake, the maximum calculated amount of Pb mg/l in lake water was found in this site (mean 0.23 mg/l) with mean pH 8.5, and mean water temperature  $23^{\circ}\text{C}$  in the presence of mean water current  $0.106 \text{ m/sc}^{-1}$ . Site 3 was selected from all sites thought to be the purest site at the area, the high concentration of Pb and other heavy metals which were recorded will bring into mind a big question mark about the disposal of gas factories

nearby. Data illustrated in Fig. (11) showed that the maximum calculated concentration of Pb in sediment was found in site 5 (Bahr El-Baqar drain-Pb mean  $100 \mu\text{g/g}$ ) with water current found at  $0.08 \text{ m/sc}^{-1}$ . It is probably due to the deposition of the industrial disposal into the bottom of the drain for a long time. These results are in agreement with Ali and Abdel-Satar (2005) attributed the increase of heavy metal concentrations in the water during to the release of heavy metals from the sediment to the overlying water under the effect of both high temperature and a fermentation process resulting from the decomposition of organic matter.



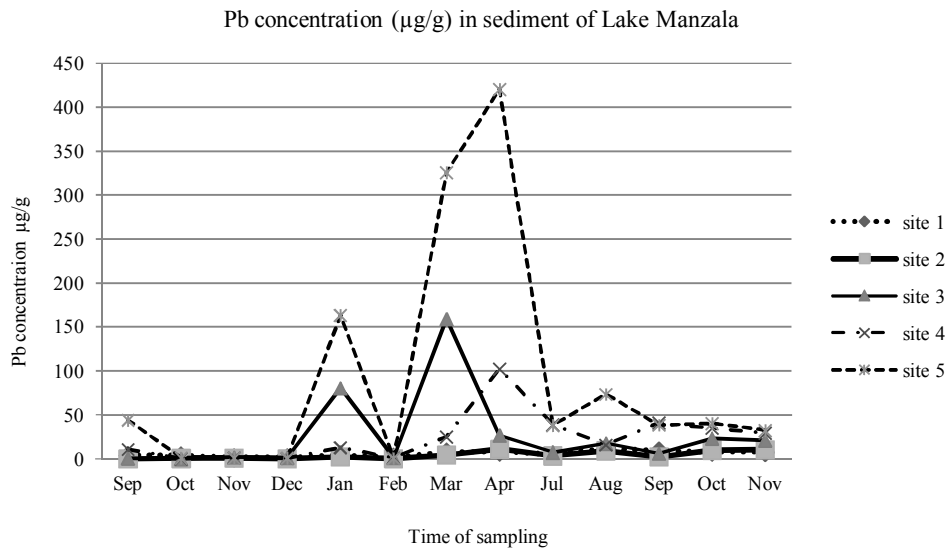
**Figure 10: The values of Pb concentration (mg/l) in water of Lake Manzala at different study sites**



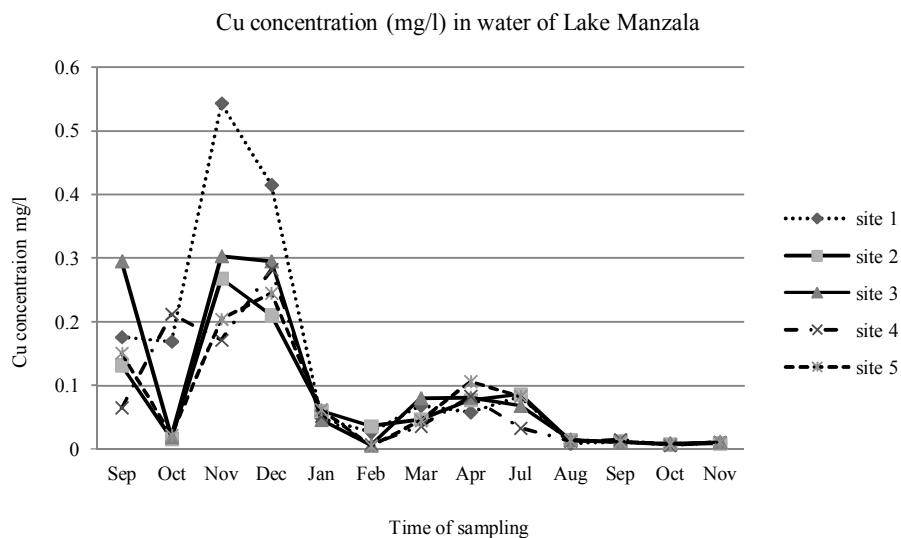
**- Cu Concentration**

Results were illustrated in Fig. (12), show that site 1 was still contained the higher total concentration of Cu in water (mean 0.15 mg/l) at mean values of pH 8.12 , water temperature 20.8 °C in the presence of water current 0.029 m/sc<sup>-1</sup> as a result of its exposed to the nearby direct industrial disposal. From Fig.(13), results show that the maximum calculated amounts of Cu in soil sediment samples were found in site 5 (mean 32.5

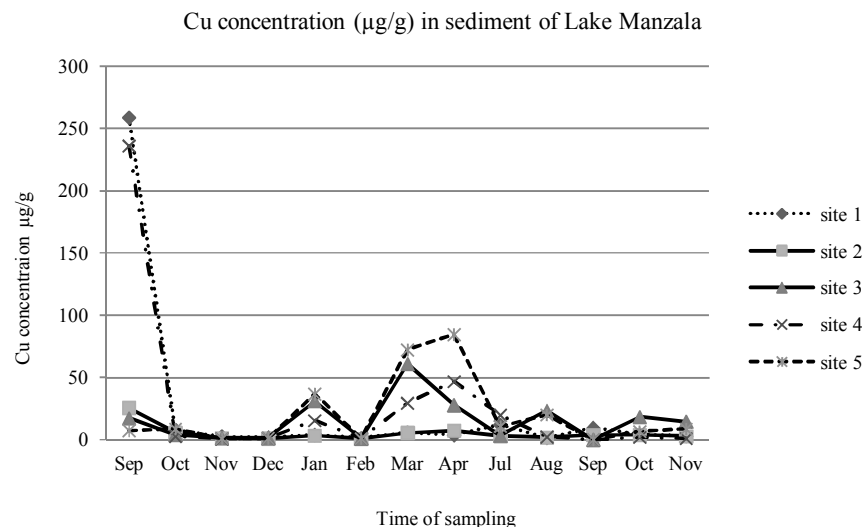
µg/g) with water current found at 0.016 m/sc<sup>-1</sup>. Again, it is probably due to the industrial disposal spilled into the drain and settled down at the bottom of the drain. The pervious results are disagreement with Adel-Hamid and El-Zareef (1996) they found lower Cu concentrations in lake water (0.01-0.02 mg/l), and the results are agreement with Abdel-Bakey *et al.*, (1998) and Ali and Abdel-Satar (2005) they recorded higher values of Cu in Lake Manzala water and sediment.



**Figure11: The values of Pb concentration (µg/g dry wt.) in surfacial sediment of Lake Manzala at different study sites**



**Figure 12: The values of Cu concentration (mg/l) in water of Lake Manzala at different study sites**



**Figure13:** The values of Cu concentration ( $\mu\text{g/g}$  dry wt.) in surfacial sediment of Lake Manzala at different study sites

### 3.3.2 Heavy metals concentrations in fish flesh

**Table 2:** The concentration of heavy metals in dry fish flesh  $\mu\text{g/g}$  dry wt. in fish samples (*Oreochromis niloticus*) were taken randomly from Lake Manzala

Heavy metals	Concentration in fish flesh $\mu\text{g/g}$ dry weight								
	PL*	February	March	April	July	August	September	October	November
Mn	2.0-9.0	19.98	19.78	24.42	6.820	22.57	1.205	16.07	6.884
Cd	0.10	0.120	0.090	0.080	0.070	0.080	0.250	0.072	0.076
Zn	60.0	98.86	101.7	93.50	66.81	80.12	18.00	27.18	52.26
Pb	0.14	4.240	3.525	4.076	4.310	3.790	5.710	2.836	1.976
Cu	3.00	0.745	1.135	3.450	1.995	1.230	0.050	3.010	2.740

\*PL: Permissible limits (wet wt.) according to FAO/WHO (1999).

Results in Table (2), show that: In the case of Cd almost fish samples give lower value comparing with the permissible limits (FAO/WHO is  $0.1 \mu\text{g/g}$ ), except the one sample, which taken in September. The Cd value of this sample was  $0.25 \mu\text{g/g}$  of dry weight. On the contrary, in the case of determine Zn in fish flesh, almost fish samples were gave higher values (98.86, 101.7, 93.50, 66.81 and 80.12) comparing with permissible limits ( $60 \mu\text{g/g}$ ), but the sample taken in September gave value under the permissible limits ( $18.00 \mu\text{g/g}$ ). The Pb was measured in different location in Lake Manzala. All fish samples were contaminated with Pb in level up of the permissible limits ( $0.14 \mu\text{g/g}$ ), the highest value was recorded in September ( $5.710 \mu\text{g/g}$ ) and the lowest value was recorded in November ( $1.97 \mu\text{g/g}$ ) and the mean value is  $3.8 \mu\text{g/g}$  flesh. In the case of Cu almost all fish samples were recorded around the limit of standard ( $3 \mu\text{g/g}$ ), but the fish samples taken in April and October were recorded the highest value ( $3.450$ ;  $3.010 \mu\text{g/g}$ ) comparing with Permissible limits ( $3.00 \mu\text{g/g}$ ). Normally the fish flesh containing a great amount of Mn which differ from kind to kind as well as differ by fish age. The maximum values of Mn were found in all samples except samples

collecting in July, September and November ( $6.820$ ;  $1.205$  and  $6.884 \mu\text{g/g}$  respectively) comparing with Permissible limits ( $2.0- 9.0 \mu\text{g/g}$ ). These results are in agreement with Jobling (1995) attributed the high accumulation of heavy metals in fish tissues to the metallothionein proteins which are synthesized in liver tissues when fishes are exposed to heavy metals and detoxify them. These proteins are thought to play an important role in protecting them from damage by heavy metal toxicants. Similar observations were reported by many studies carried out with various fish species (Guerrin *et al.*, 1990 and Saeed and Sakr, 2008).

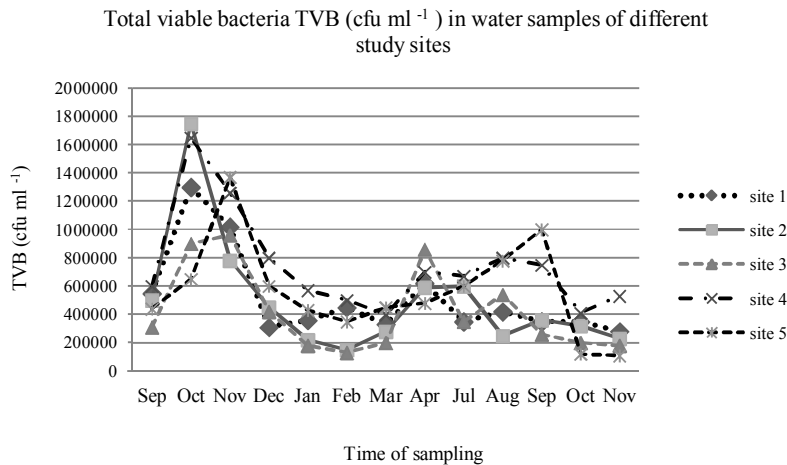
### 3.2 Microbiological parameters:

#### 3.2.1 Bacterial load (total viable bacteria TVB and faecal coliforms FCB) in water of Lake Manzala

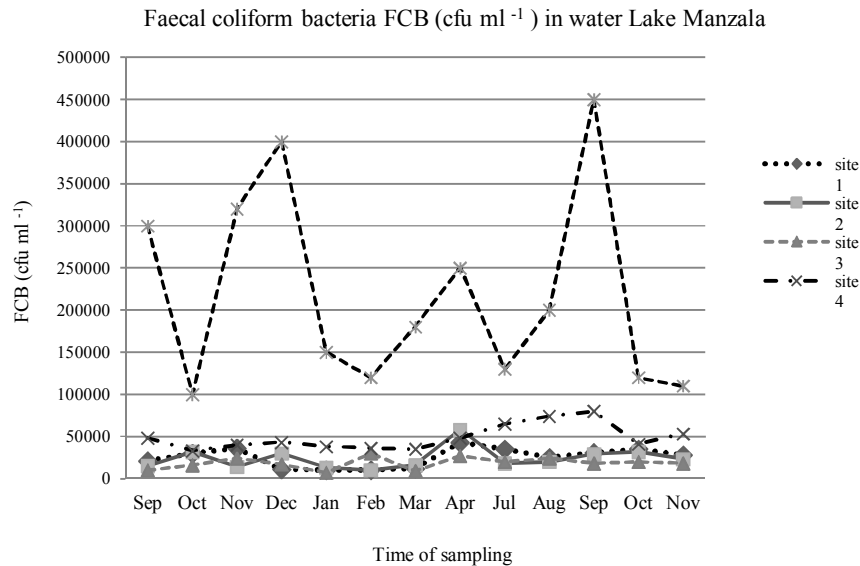
The maximum calculated values of total count viable bacteria (TVB) in water samples were found in site 4 [El-Bashtier], when mean pH is 7.8 and mean water (temperature  $22.3 \text{ }^\circ\text{C}$  in the presence of water current of  $0.016 \text{m/Sc}^{-1}$ ). It is probably due to the effect untreated waste water at the drain which its spill near site 4 Fig. (14). While the maximum calculated values of total count faecal coliform bacteria (FCB) in water samples

were recorded clearly in site number 5 [Bahr El-Baqar drain] at mean values of pH 7.5 and water temperature 22.9 °C in the presence of water current at rate 0.095m/Sc<sup>-1</sup>. It is probably due to the effect untreated waste water at the drain. Although site 3 is near the connection between Sea and lake but the total number of FCB is too high. It is

probably due to the waste water station disposal which spills its untreated waste water directly to the lake near this site. The previous results are in agreement with Uddin *et al.*, (1990); Fernandes *et al.*, (1999) and Al-Harbi (2003) they found the bacteria load in water samples increased by increasing water temperature and organic matter.



**Figure14: Total viable bacteria (TVB) count by cfu ml<sup>-1</sup> in water samples were collected from different study sites in Lake Manzala**



**Figure15: Faecal coliform bacteria (FCB) count by cfu ml<sup>-1</sup> in water samples were collected from different study sites in Lake Manzala**

**3.2.2 Bacterial load (total viable bacteria TVB and faecal coliforms bacteria FCB) in various fish organs of *Oreochromis niloticus* taken randomly from study sites**

The results in Table (3) show that all fish samples which were collected randomly from different sites of Lake Manzala contaminated by a high rate especially two samples in February and November. The mean load of bacteria in these periods were found at high level ( $1.59 \times 10^7$ ,  $1.34 \times$

$10^7$  cfu g<sup>-1</sup> respectively) comparing with Egyptian specification (50 cfu g<sup>-1</sup>). This result gives a good indication about the effect of untreated waste water spilled on the aquatic life in Lake Manzala. Similar results are agreement with Chowdhury *et al.*, (1989) and Al-Harbi (2003) they observed the bacterial load in fish intestine during the period of study was correlated with the bacterial levels in the aquatic environment.

Data presented in Table (4) show that the count of faecal coliforms bacteria (FCB) in various organs of fish samples. All tested fish samples were contaminated by FCB with high level of contamination. The highly mean loads of bacteria in these samples were found in August by rate  $1.09 \times 10^7$  cfu g<sup>-1</sup> comparing with Egyptian specification (50 cfu g<sup>-1</sup>). This results were emphasizes on a large amounts of domestic and agriculture waste water disposal directly or indirectly in the lake without any treatments. Leung

*et al.*, (1992); Fernandes *et al.*, (1997) and Pullela *et al.*, (1998) are observed similar results in the faecal coliforms in tilapia (*Oreochromis niloticus*) intestine, and they recorded faecal coliforms bacteria represent a potential problem in pond effluent management. Fish and fish products have long been considered a vehicle of food-borne bacterial parasitic infections leading to human illness. Further research is needed to elucidate the behavior of bacterial contaminants in tilapia fish as well as in the pond ecosystem in Lake Manzala.

**Table 3: Total viable bacteria TVB count ( cfu g<sup>-1</sup>) in various fish organs (*Oreochromis niloticus*)**

Time of sampling	Total viable bacteria count (cfu g <sup>-1</sup> )			
	Fish organs			
	Gill	Muscle	Intestine	Mean load
February	2.78± 0.18× 10 <sup>4</sup>	ND	3.19± 0.21× 10 <sup>7</sup>	1.59× 10 <sup>7</sup>
March	3.40± 0.23× 10 <sup>4</sup>	ND	1.44± 0.09× 10 <sup>7</sup>	7.21 × 10 <sup>6</sup>
April	1.44±0.09× 10 <sup>4</sup>	ND	1.13±0.07× 10 <sup>5</sup>	6.37× 10 <sup>4</sup>
July	3.29± 0.23× 10 <sup>2</sup>	ND	1.03±0.09× 10 <sup>5</sup>	5.16× 10 <sup>4</sup>
August	3.60± 0.24× 10 <sup>3</sup>	ND	1.23± 0.08× 10 <sup>6</sup>	6.16× 10 <sup>5</sup>
September	3.19± 0.21× 10 <sup>2</sup>	ND	2.16± 0.14× 10 <sup>5</sup>	1.08× 10 <sup>5</sup>
October	6.08± 0.41× 10 <sup>2</sup>	ND	1.95± 0.13× 10 <sup>5</sup>	9.78× 10 <sup>4</sup>
November	1.54± 0.10× 10 <sup>3</sup>	ND	2.68± 0.18× 10 <sup>7</sup>	1.34× 10 <sup>7</sup>

- Mean ± stander deviation

- ND: Not detected in test

**Table 4: Faecal coliforms bacteria FCB (cfu g<sup>-1</sup>) in various fish organs (*Oreochromis niloticus*)**

Time of sampling	Faecal coliforms FCB (cfu g <sup>-1</sup> )			
	Fish organs			
	Gill	Muscle	Intestine	Mean load
February	ND	ND	2.17± 0.16× 10 <sup>6</sup>	1.08× 10 <sup>6</sup>
March	1.13±0.07× 10 <sup>4</sup>	ND	1.33± 0.08× 10 <sup>6</sup>	6.70× 10 <sup>5</sup>
April	1.03±0.09× 10 <sup>4</sup>	ND	1.55± 0.11× 10 <sup>6</sup>	7.80× 10 <sup>5</sup>
July	1.23± 0.08× 10 <sup>4</sup>	ND	2.37±0.16× 10 <sup>5</sup>	1.24× 10 <sup>5</sup>
August	2.16± 0.14× 10 <sup>3</sup>	ND	2.19± 0.21× 10 <sup>7</sup>	1.09× 10 <sup>7</sup>
September	1.95± 0.13× 10 <sup>4</sup>	ND	1.43± 0.09× 10 <sup>7</sup>	7.15× 10 <sup>6</sup>
October	2.68± 0.18× 10 <sup>4</sup>	ND	2.27± 0.16× 10 <sup>6</sup>	1.14× 10 <sup>6</sup>
November	1.23± 0.08× 10 <sup>4</sup>	ND	3.32± 0.08× 10 <sup>6</sup>	1.66× 10 <sup>6</sup>

- Mean ± stander deviation

- ND: Not detected in test

#### 4. Conclusion

Lake Manzala is a very important lake in Egypt due to its dimensions and economic activity. The results of the present study clearly demonstrate that Lake Manzala is highly contaminated with Mn, Cd, Zn, Pb and Cu due to the continuous discharge of different pollutants into it. Metal contamination in water, sediment, and fish organs followed the order of Zn > Mn > Pb > Cu > Cd. The highest metal concentrations were found in fish tissues from the most contaminated site, showing that metal accumulation in *Oreochromis niloticus* reflects the degree of water pollution. All the five sites were contaminated with high amount of TVB and FCB in water which is an indicator of untreated waste water which spilled directly or indirectly to the lake. The most alarming result was found when analyzing fish; all the fish samples were contaminated on surface and internally with very high amounts of TVB and FCB at gill and intestine. This confirms that lake fish is highly polluted and dangerous for human health. The results of this study supplied valuable information on the level of metal contamination in Lake Manzala. Great efforts

and cooperation between different authorities are needed to protect the lake from pollution and reduce environmental risk. This can be achieved by treatment of the agricultural, industrial, and sewage discharge. Regular evaluation of pollutants in the lake is also very important.

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