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Monitoring of Heavy Metals in Major Drains and Farms at Fayoum Government and Treatment by Pumpkin and Eggplant

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ABSTRACT

This study was carried out in three different farms and drainages at EL-Fayoum Government in Egypt. Five heavy metals (Zn, Cd, Pb, Fe and Cu) were addressed in farms and drain water. The organs (gills, skin, muscles and liver) and blood of selected fish; gathered from various farms and drains, were examined to measure the heavy metals' residue. The results showed that, iron was the most accumulated metal in the three drains and farms, while zinc was the most accumulated metal in the Nile tilapia blood. For water treatment, pumpkin showed high removal of Zn (65%) and Pb (50%), while eggplant gave maximum removal values of Fe, Cd and Cu (88%, 78% and 77%, respectively). In the current study, investigation of some biochemical blood parameters were also determined, such as alanine aminotransferase, aspartate aminotransferase enzyme, urea, creatinine, random blood sugar, creatinine kinase, alkaline phosphatase and thyroid-stimulating hormone, thyroxine and triiodothyronine, growth hormone and cortisol hormones.

INTRODUCTION

Drain water is the removal of a surface water from an area with high amount of water whether naturally or industrially originated. Water is carried to outlet by main drains collecting water from the laterals. Sub mains branched from the main drain are used to collect water **(Dalrymple, 2015)**. In Egypt, the annual supply of water is about 57.7 billion cubic meters collected from the Nile, rainfall along the Mediterranean coast and the deep groundwater as well. However, the estimated used water for farms, industries, and people is around 72.4 BCM **(Barnes, 2014)**. It is noteworthy to mention that, most of the heavy metals are bound to particles in sediment, though a small quantity is dissolved in water and can spread widely mounting the atmost top of the food chain **(El-Nemeki *et al.*, 2008)**. Furthermore,wells, rivers, dams and large ponds are good sources of water for fish farm ponds **(Gabbadon & De Souza, 2008)**. Hence, aquatic organisms are exposed to high levels of heavy metals. Since fish are relatively sensitive to changes in their surroundings environment, fish health would indicate a good sign of the health state of a specific aquatic ecosystem. Remarkably, poisonous effects of pollution appear on cellular or tissue level early before significant changes can be observed in fish behavior or external appearance **(Abdel-Warith *et al.*, 2011)**. Moreover, blood is a path physiological reverse of the all body parts. Consequently, blood parameters are important in examining the structural and functional state of fish insecure to toxicants. Eminently, variation in the biochemical blood factors indicate changes in metabolism and biochemical processes of the organism due to the effects of various pollutants **(Adhikari *et al.*, 2004)**. The levels of serum enzymes have been widely used as indicators of health for different animals like fish. Hence, the evaluation of those blood components may help to determine the influence of stressors on the homeostasis of those animals, especially fish enabling the detection of early conditions that could be affecting the production performance **(Cnaani *et al.*, 2004)**. It was noticed that, metal ions can be joined to food chains and fixed in aquatic organisms to a level that affects their physiological state. In this essence, zinc, copper and iron have a biochemical role in the life processes of all aquatic organisms especially fish as they are essential in the aquatic environment in little amounts **(Saeed & Shaker, 2008)**. Pollution of the aquatic environment is a sign of danger and may cause problems around the world. The highest amount of industrial, agricultural and commercial chemicals spreading into the aquatic environment have various dangerous impacts on aquatic organisms. Thus, heavy metals' residue of aquatic system has gained the interest of several researchers **(Yacoub & Gad, 2012)**. Residue of heavy metals varied due to species, ages, sex and organs. Liver and gills have metabolic activity where high levels of metal are accumulated in fish, while in muscles the metabolic activity is relatively low accumulating less level of heavy metals **(Younis *et al.*, 2002)**. Eminently, residue activities in fish tissues depends on metal condensation (i.e: metal concentration), time of exposition, metal uptake, environmental conditions (water nuclear fuels, temperature, pH, hardness, salinity), fish age and feeding habits. The tiny size of heavy metals are various in affinity to penetrate fish tissues allowing toxic metals to be released into liver, kidney, gills and fish muscles. Comparing to the other mentioned tissues, fish muscles usually include the lowest levels of metals. Residue of heavy metals in different organs of fish may cause structural damage and commonly lead to functional disturbances **(Jezierska & Witeska, 2006)**. Therefore, the aim of the present study was to follow up the effect of different sources of drainage on fish farms in Fayoum Governorate by collecting water and fish samples from farms and measuring five heavy metals (Fe, Cu, Zn, Cd and Pb). The accumulation of those metals in water, different fish organs and fish blood were examined. Moreover, low cost adsorbent such as eggplant and *Cucurbita pepo* were used to treat heavy metals in the highly polluted drain. Moreover identification of biochemical changes like glucose, liver enzymes, kidney functions, triglycerides, cortisol hormone, growth hormone and thyroid hormones was also studied.

MATERIALS AND METHODS

The experiment was carried out in Shakshouk Fish Research Station, EL-Fayoum Governorate, National Institute of Oceanography and Fisheries, Egypt.

Collection of Water and Fish samples

Water samples were collected from three major different drainages at EL-Fayoum Governorate (Dier El-Berka , EL-Wadi and EL-Bats drains) and three different commercial farms where those drains pour into (EL-Gilani, Locanda and EL-Bats farms, respectively) from middle site. Fish samplesof the Nile tilapia (*Oreochromis niloticus*) were collected from three different commercial farms (EL-Gilani, Locanda and EL-Bats) with weights ranging from 170-230 grams and lengths from 20- 25 cm.

Heavy metals contamination

Heavy metal determination (Fe, Pb, Zn, Cu and Cd) in water samples was determined using Atomic Absorption (Perkin Elmer 3110 USA) with graphite atomizer HGA-600 at Soil and Water Laboratory, Faculty of Agriculture, Fayoum University.

Treatment of water drainage

Water samples were collected from EL-Bats drain from middle site for treatment using two adsorbent pumpkin (*Cucurbita pepo*) and eggplant (*Solanummelongena)*

Preparation of pumpkin (*Cucurbita pepo*)

A pumpkin is a cultivar of the squash plant, most commonly of *Cucurbita pepo*, it is round, with smooth, slightly ribbed skin, and deep yellow to orange coloration. Pumpkin is easily available at the local market and was purchased and prepared for treatment of heavy metals. The seeds were sun dried for 2-3 days followingthe method of **Deepthi** (**2017**). The dried seeds were pulverized into a fine powder using a pestle and mortar (Fig. 1). The required dosage (0.5 g/l) of pulverized seed material was made into a paste using a small amount of water mixed and shaken for 1 min to activate the coagulant properties of the seed at stilling time of 30 min.

Preparation of eggplant peel adsorbent

The eggplant was collected from the local market and prepared for treatment of heavy metals. The outer peel was removed then the waste peel was immersed in an HCl solution (0.01 M) to remove other impurities for 24 h. After HCl treatment, the waste eggplant peel was washed with D.W. and then obscure in 1 M NaOH solution for 48 h **(Min *et al.*, 2004)**. Next, it was rinsed with D.W. and heated in a temperature of 60°C for 30 hours in a drying oven. The dried adsorbents were ground using grinding machine and sieved with standard mesh size into sizes of 200 mm (Fig. 1) then, 4 g/l of powder was used as dosage in an optimum time of 110 min for experiment.

pumpkin adsorbent powder *eggplant adsorbent powder*

Fig. 1. Pumpkin and Eggplant Adsorbent Powders.

Blood analysis

Blood samples were taken from the caudal vein of un anaesthetized fish, 1-3 ml of blood was collected from each individual in a clean centrifuge tube. Serum was then separated by centrifuge at 3000 r.p.m for 15 min to be used for estimation of glucose levels, creatinine kinase, alkaline phosphatase, urea, creatinine, AST, ALT , cortisol hormone, growth hormone, thyroid hormones (TSH, T3, T4) and heavy metals levels (cadmium, zinc, iron, lead and copper). Serum glucose concentration was determined according to the method of **Trinder (1969)**. Serum ALP was carried out according to **Tietz *et al*. (1983)**. Serum CK was carried out according to **German clinical chemistry society (1977**). Serum cortisol was formed according to **Tunn *et al.* (1990)**. Serum T3 was carried following the descriptions of **Wild (1994)**, serum T4 was carried out foolowing **Chopra (1972)**, serum TSH was formed using the steps of **Morimoto and Inouye (1997)**. In addition, growth hormone in plasma using ELISA Kit was determined according to **Baum *et al*. (1998)**. Samples for heavy metals analysis were submitted to Analytical Toxicology Laboratory Services and the analysis was done according to the procedures of **Gajek *et al*. (2013)** by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Accumulation of heavy metals in different fish organs

Fish specimens were transferred to beakers and placed in a drying oven thermostatically regulated at 105°C overnight. Representative samples (10 g fresh muscles, 3g for liver, 10g for gills and 3g for skin) of dry weight were taken from the fish specimens. The samples were digested according to the method described by **Goldberg (1963)**.

Statistical analysis

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2008) 17, released version.

RESULTS

Heavy metals concentrations in three different water drainages before treatment

Figs. 2 and 3 show that, there was a significant decrease of Zn and Cd with *P*-value (0.02 , 0.02 respectively) in EL-Wadi drainage and mean value ± S.D of Zn levels in EL-Bats drain with *P*- value (0.001) when compared to Dyer EL-Berka drainage and *P**- value of 0.02 and 0.01 when compared to EL-Wadi drainage.

Additionally, a significant increase of Fe and Cu was detected in EL-Wadi and EL-Bats drains with *P*-value of 0,002, 0.002 and 0.003, 0.003, respectively when compared to Dyer EL-Berka drainage, while *P**- value of 0.5, 0.01 was observved when compared to EL-Wadi drainage. Moreover, Cd recorded a significant increase of mean value ± S.D in EL-Bats drains with a *P*-value of 0.02 when compared to Dyer EL-Berka drainage, and a *P**-value of 0.01 when compared to EL-Wadi drainage. But, a non-significant variation of Pb was recorded in EL-Wadi and EL-Bats drains when compared to Dyer EL-Berka drainage.

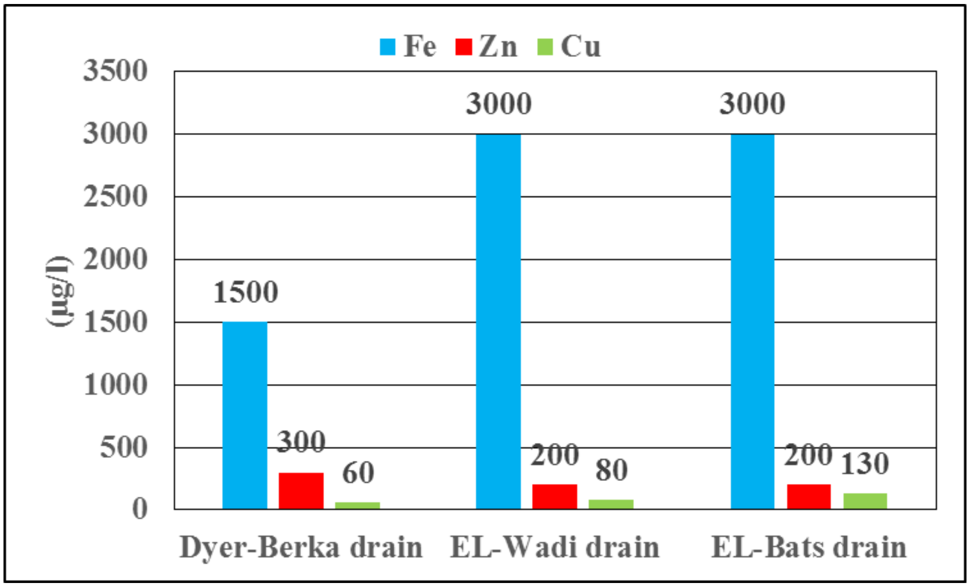

Fig. 2. Fe, Zn and Cu Concentrations (µg/l) in Three Different Water Drainages.

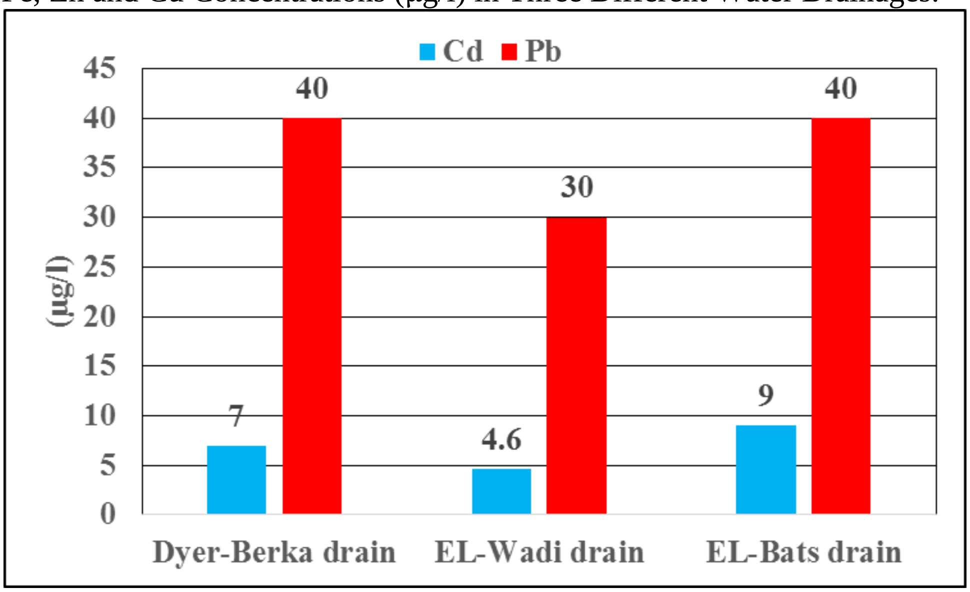

Fig. 3. Cd and Pb Concentrations (µg/l) in Three Different Drainages.

Heavy metal concentrations in the water of three different farms

As shown in Fig (4, 5), a significant decrease was detected in the levels of Zn and Fe in Locanda and EL-Bats farms with *P*-value of 0.001, 0.001 for Zn, and <0.001, <0.001 for Fe, respectively. However, an increased value of Fe was detected in EL-Bats when compared to EL-Galini farm recording *P**- value <0.001, 0.01 when compared to Locanda farm.

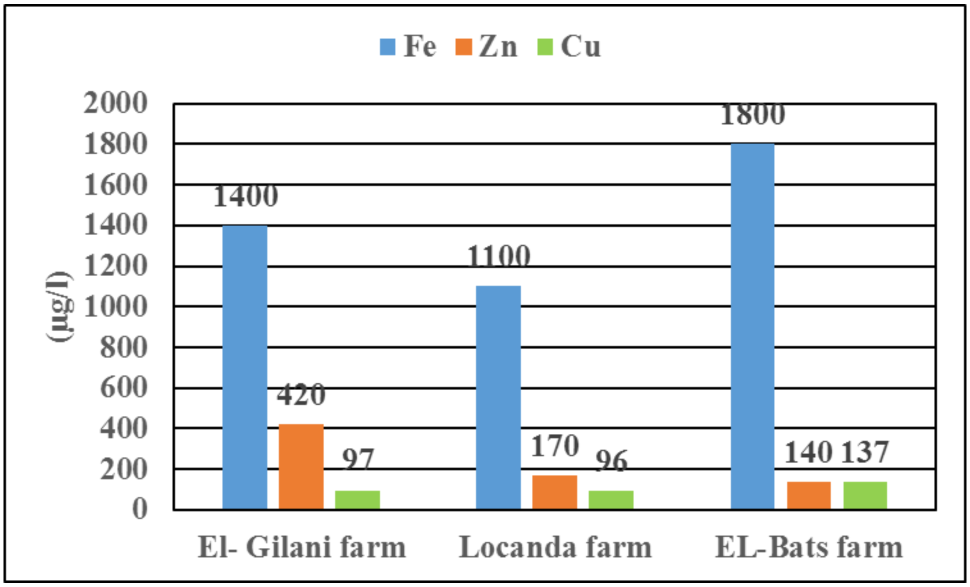

Fig. 4. Fe, Zn and Cu Concentrations in Water of Three Different Fish Farms.

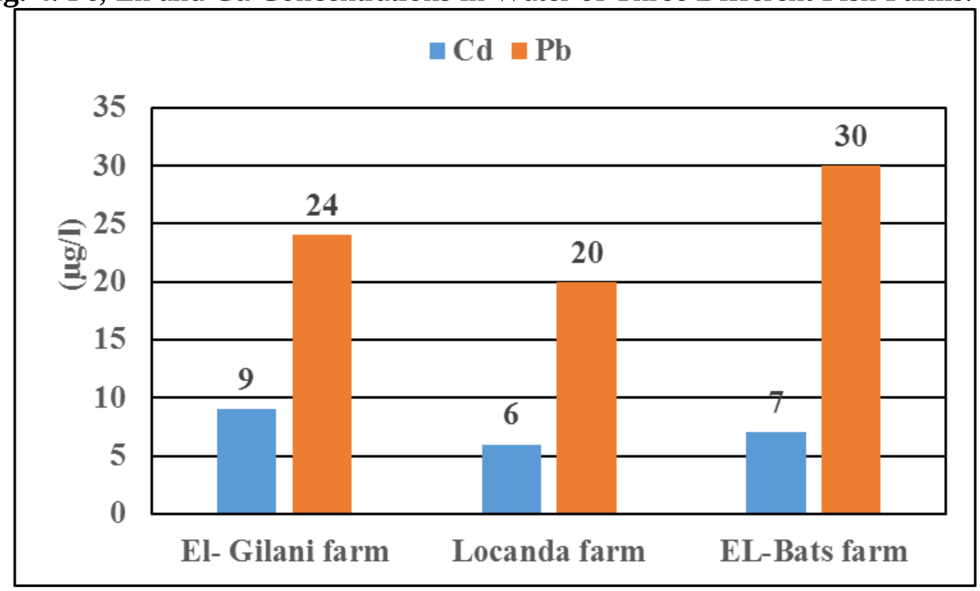

Fig. 5. Cd and Pb Concentrations in Water of Three Different Fish Farms.

On the other hand , there was a significant increase of levels of Cu in EL-Bats farms with a *P*-value of 0.004 and *P**- value of 0.001 when compared to Locanda farm. In addition, a non-significant variation was recorded in Locanda farm when compared to EL-Galini farm. While, non-significant levels of Cd and Pb were dedermined in both Locanda and EL-Bats when compared to EL-Galini farm.

Treatment by pumpkin

The removal of different metals using prepared adsorbent from pumpkin is explained in Table (1). It is clear that, this adsorbent showed a good removal of all the metal at dosage of adsorbent (0.5 g/l) contact time 30 min and temperature 25°C. Table (1) records a significant decrease of levels of Zn, Cu, Fe and Pb with *P*-value of 0.01, 0.001, <0.001 and 0.05, respectively in EL-Bats drain under effect of treatment by pumpkin when compared to EL-Bats drain before treatment. But, Cd levels showed non-significant variation in EL-Bats drain under effect of treatment by pumpkin when compared to EL-Bats drain before treatment. It was clear from the obtained results that, the order of removal for the different heavy metals followed the succeeding order: Fe> Cd> Zn> Cu>Pb. For Fe,the maximum removal was 77% , while the minimum was recorded for Pb recording a removal value of 50%.

Treatment by eggplant

The prepared adsorbent from eggplant was also examined for the removal of heavy metals from the polluted drain Table (2). The results showed that this adsorbent give high percent of metal removal at an adsorbent dose of 4 g/l, contact time of 110 min and temperature of 25°C. It was noticed from the results that are presented in Table (2) that, levels of Zn, Cu, Fe and Cd recorded a significant decrease with *P*-value of 0.02, 0.001, 0.001 and 0.01, respectively in EL-Bats drain under effect of treatment by eggplant when compared to EL-Bats drain before treatment. Nevertheless, the Pb levels of mean value ± S.D showed a non-significant variation in EL-Bats drain under effect of treatment with eggplant when compared to EL-Bats drain before treatment. Consequently, the order of removal for the different heavy metal were as follows: Fe> Cd> Cu> Zn and Pb (Zn and Pb showed equal removal percentage). The maximum removal 88% for Fe, while the minimum was 25% for Zn and Pb under effect of treatment with eggplant.

Table 1. Heavy Metals Concentrations (µg/l) in EL-Bats Water Drainage before and after Treatment with pumpkin.

Groups / Heavy metals		Before	After	percent of removal %
Zinc (µg/l)	Range Means±SD *p*-value	200-205 200±3.5	70-75 70±3.5 0.012	65
Copper (µg/l)	Range Means±SD *p*-value	130-135 130±3.5	50–54 50±2.8 0.001	62
Iron (µg/l)	Range Means±SD *p*-value	3000-3010 3000±7.0	700-720 700±14.1 <0.001	77
Lead (µg/l)	Range Means±SD *p*-value	40-45 40±3.5	20-24 20±2.8 0.03	50
Cadmium (µg/l)	Range Means±SD *p*-value	9-10 9±0.3	3-5 3±1.4 0.09	67

- Data are represented as means ± SD; - P-value :-when different groups compared to EL-Bats drain; p > 0.05 is non- significant - p ≤0.05 is significant.

Table 2. Heavy Metals Concentrations (µg/l) in EL-Bats Water Drainage before and after Treatment with Eggplant.

Heavy metals \ Groups		Before	After	percent of removal %
Zinc (µg/l)	Range Means±SD *p*-value	200-205 200±3.5	150-154 150±2.8 0.02	25
Copper (µg/l)	Range Means±SD *p*-value	130-135 130±3.5	31-34 31±2.1 0.001	77
Iron (µg/l)	Range Means±SD *p*-value	3000-3010 3000±7.0	354-355 355±0.7 0.001	88
Lead (µg/l)	Range Means±SD *p*-value	40-45 40±3.5	30-34 30±2.8 0.08	25
Cadmium (µg/l)	Range Means±SD *p*-value	9-10 9±0.3	2-3 2±0.7 0.01	78

- Data are represented as means ± SD; - P-value :-when different groups compared to EL-Bats drain; p > 0.05 is non- significant
- p ≤0.05 is significant.

Biochemical parameter levels of fish blood in three different farms

Table (3) shows a significant increase of RBS and ALP with *P*-value of 0.004, 0.002 for RBS and 0.01, 0.001 for ALP, respectively in both Locanda and EL-Bats farms when compared to EL-Galini farm and *P**-value of 0.002 and 0.001 when compared to Locanda farm.

Table 3. Biochemical Parameters in Fish Blood of Three Different Farms.

Biochemical Parameters \ Groups		EL-Galini Farm	Locanda Farm	EL-Bats Farm
RBS (mg/dl)	Range Means±SD *p*-value *P**-value	61.0-61.23 61.12 ± 0.16	108.0-108.09 108.46 ± 0.65 0.004	82.0-82.52 82.26± 0.36 0.002 0.002
Urea (mg/dl)	Range Means±SD *p*-value *P**-value	16.4-16.4 16.4±0.01	13.04-13.04 13.04±0.01 <0.001	2.6-23.5 23.05±0.63 0.04 0.03
Creatinine (mg/dl)	Range Means±SD *p*-value *P**-value	0.86-0.86 0.86±0.01	0.64-0.65 0.64±0.01 0.02	0.60-0.70 0.65±0.07 0.05 <0.001

ALT (U/L)	Range Means±SD *p*-value *P**-value	21.2-21.6 21.41±0.29	123.56-123.58 123.57±0.01 0.01	16.0-18.52 18.26±0.36 0.01 0.002
AST (U/L)	Range Means±SD *p*-value *P**-value	340.0-340.0 340.00±0.01	154.27-154.4 154.30±0.14 <0.001	111.8-111.9 111.8±0.07 <0.001 <0.001
Alkaline phosphatase (ALP) (IU/L)	Range Means±SD *p*-value *P**-value	30.0-30.9 30.4±0.64	81.51-81.52 81.52±0.01 0.01	105.5-105.6 105.65±0.21 0.001 0.001
Creatinine kinase (CK) (mg/dl)	Range Means±SD *p*-value *P**-value	20952-20952.1 20952.52±0.7	7215.9-7216.0 7257.90±0.07 <0.001	9157.8-9158.0 9157.90±0.14 <0.001 <0.001

-Data are represented as means ± SD of 3 farms; P-value :- when different groups compared to EL-Galini farm.; P*-value :- when different groups compared to Locanda farm.; . p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation

Additionally, there was a significant decrease of of creatinine, CK and AST with *P*-value of 0.02, 0.05 for creat., <0.001, <0.001 for CK and <0.001, <0.001 for AST, respectively in both Locanda and EL-Bats farms when compared to EL-Galini farm, recording a *P**-value (<0.001) when compared to Locanda farm. On other hand, there was a significant decrease of urea in Locanda farm with *P*-value (<0.001) but, a significant increase with *P*-value (0.04)was detected in EL-Bats farm when compared to EL-Galini farm and *P**-value (0.03) was regarded when compared to Locanda farm. The current results detected a significant increase of ALT in Locanda farm with a *P*-value of 0.01. Meanwhile, a significant decrease with a *P*-value of 0.01 was recorded in EL-Bats farm when compared to EL-Galini farm and *P**-value (0.002) as detected when compared to Locanda farm.

Hormone levels in fish blood of three different farms

Table (4) reveals that, a significant increase of cortisol in Locanda farm with *P*-value (<0.001) was recorded, while a significant decrease with a *P*-value of 0.01 was observed in EL-Bats farm when compared to EL-Galini farm, and recording a *P**-value of 0.01 when compared to Locanda farm. Moreover, there was a non-significant variation of GH in Locanda farm while a significant decrease was determined with a *P*-value of 0.01 in EL-Bats farm when compared to EL-Galini farm. On the other hand, a significant increase of levels of T3 was noticed in Locanda farm with *P*-value (0.05) and *P**-value (0.01) when compared to Locanda farm and non-significant variation in EL-Bats farm when compared to EL-Galini farm. Additionally, there was non-significant variation of levels of T4 and TSH in both Locanda and EL-Bats farms when compared to EL-Galini farm.

Table 4. Hormone Levels in Fish Blood of Three Different Farms.

Hormones \ Groups		ELGalini Farm	Locanda Farm	EL-Bats Farm
Cortisol (mcg/dL)	Range Means±SD *p*-value *P**-value	9.8-9.9 9.85±0.07	15.8-15.9 15.85±0.07 <0.001	4.0-4.4 4.20±0.28 0.01 0.01
Growth hormone (ng/mL)	Range Means±SD *p*-value *P**-value	0.47-0.49 0.48±0.10	0.52-0.53 0.51±0.01 0.13	0.23-0.24 0.24±0.01 0.01 0.01
T3 (ng/dL)	Range Means±SD *p*-value *P**-value	7.2-9.0 8.10±1.27	19.3-19.4 19.35±0.70 0.05	6.6-7.0 6.80±0.28 0.3 0.01
T4 (ng/dL)	Range Means±SD *p*-value *P**-value	0.7-0.9 0.80±0.14	1.22-1.23 1.23±0.01 0.1	0.7-0.9 0.80±0.14 1.0 0.1
TSH (ng/dL)	Range Means±SD	0.02-0.02 0.02±0.01	0.04-0.04 0.04±0.01	0.02-0.02 0.02±0.01

-Data are represented as means ± SD of 3 farms.; P-value :- when different groups compared with EL-Galini farm.; P*-value :- when different groups compared with Locanda farm.; p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation

Heavy metal concentrations in fish blood of three different farms

Fig (6) reveals that, a significant decrease of Zn was detected in both Locanda and EL-Bats farms with a *P*-value <0.001 and 0.001, respectively when compared to EL-Galini farm, and recording a *P**-value <0.001 when compared to Locanda farm. Moreover, there was a significant decrease of Cu in Locanda farm with *P*-value of 0.01, while a significant increase was detected in EL-Bats farm with a *P*-value of 0.001 when compared to EL-Galini farm and a *P**-value of 0.003 when compared to Locanda farm. Furthermore, there was a significant increase in Fe value in Locanda farm with a *P*-value of 0.04. Simultaneously, a significant decrease was recorded in EL-Bats farm with a *P*-value of 0.001 when compared to EL-Galini farm and a *P**-value <0.001 when compared to Locanda farm. Remarkably, Cd and Pb levels were not detected in fish blood of the three different farms under study.

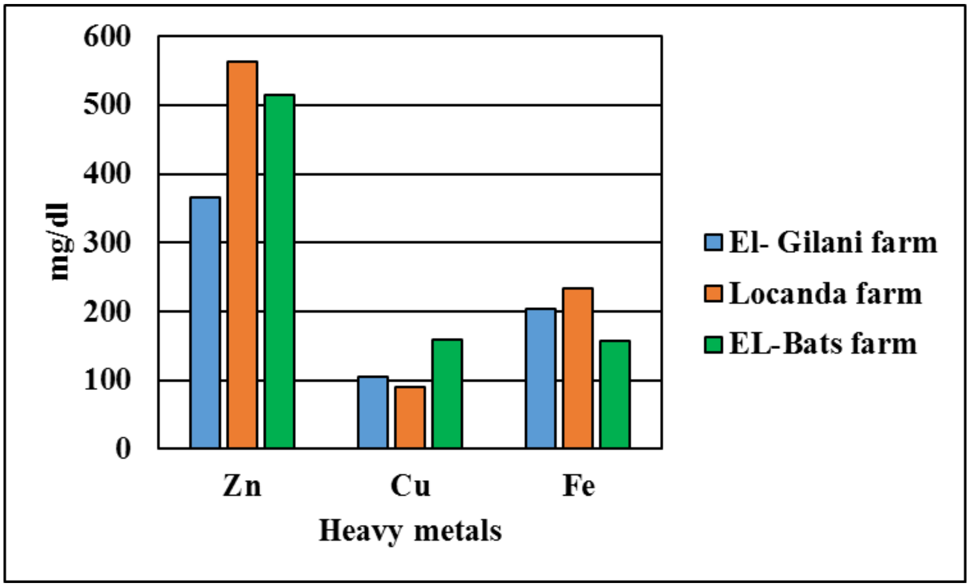

Fig. 6. Heavy Metals Concentrations in Fish Blood of Three Different Farms (mg/dl).

Table 5. Accumulation of Heavy Metals in Fish Liver of Three Different Farms (mg/ kg) .

Heavy metals \ Groups		EL-Galini farm	Locanda farm	El-Bats farm
Zinc (mg/ kg)	Range Means±SD *p*-value *P**-value	79.8-79.9 79.8±0.07	41.2-41.3 41.2±0.07 <0.001	27.8-28.0 27.9±0.14 <0.001 0.001
Copper (mg/ kg)	Range Means±SD *p*-value *P**-value	90.4-95.5 92.9±3.0	48.5-52.8 50.7±3.0 0.001	50.2-60.3 57.8±10.7 0.05 0.05
Iron (mg/ kg)	Range Means±SD *p*-value *P**-value	455.3-456.6 455.4±3.0	372.5-373.0 372.8±0.3 <0.001	255.3-262.5 258.9±5.0 <0.001 0.02
Lead (mg/ kg)	Range Means±SD *p*-value *P**-value	0.56-0.58 0.57±0.01	1.0-1.0 1.0±0.0 0.02	1.6-1.6 1.6±0.0 0.01 0.01
Cadmium (mg/ kg)	Range Means±SD *p*-value *P**-value	0.8-0.9 0.85±0.07	0.04-0.04 0.04±0.00 0.02	0.04-0.04 0.04±0.00 0.04 <0.001

-Data are represented as means ± SD of 3 farms.; P-value :- when different groups compared to EL-Galini farm.; P-value :- when different groups compared to Locanda farm.; . p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation*

Accumulation of heavy metal concentrations in fish liver of three different farms

Results recorded in Table (5) show that, there was a significant decrease of Zn , Cu, Fe and Cd in both Locanda and EL-Bats farms with *P*-value (<0.001, <0.001 for Zn), (0.007,0.05 for Cu),(<0.001, <0.001for Fe) and (0.02,0.04 for Cd) respectively when compared to EL-Galini farm recording a *P**-value (0.001, 0.05, 0.02, <0.001) when compared to Locanda farm. On the other hand , there was a significant increase of Pb in both Locanda and EL-Bats farms with *P*-value (0.02 and 0.04) when compared with EL-Galini farm and *P**-value (0.001) when compared to Locanda farm. All heavy metals under study showed variation in contamination in all three different farms in the following order Fe> Cu> Zn> Cd> Pb in EL-Galini farm. But, they were formed in the following order Fe> Cu> Zn> Pb> Cd in both Locanda and EL-Bats farms.

Accumulation of heavy metals in fish muscles of three different farms

As shown in Table (6), there was a significant decrease of Zn and Fe with *P*-value (0.001, 0.002 for Zn) and (0.003, <0.001 for Fe), respectively in Locanda and EL-Bats farms when compared to EL-Galini farm and *P**-value (0.002) when compared to Locanda farm. Additionally, there was a non- significant variation of Cu in both Locanda and EL-Bats farms when compared to EL-Galini farm. But, neither Cd nor Pb was detected in fish muscle at the three different farms under study. All heavy metals under study showed variation in contamination in all three different farms in the following order Fe> Cu> Zn.

Table 6. Accumulation of Heavy Metals in Fish Muscles of Three Different Farms (mg/ kg).

Heavy metals	Groups	EL-Galini Farm	Locanda Farm	EL-Bats Farm
Zinc (mg/ kg)	Range	47.7-47.9	34.3-34.4	18.9-18.9
	Means±SD	47.8±0.14	34.3±0.07	18.9±0.0
	p-value		0.001	0.002
	*P**-value			0.002
Copper (mg/ kg)	Range	6.9-8.7	3.7-9.2	4.1-4.4
	Means±SD	7.55±1.6	6.45±3.8	4.25±0.21
	p-value		0.7	0.2
	*P**-value			0.5
Iron (mg/ kg)	Range	1181.2-1197.2	221.9-225.7	238.9-253.7
	Means±SD	1189.2±11.3	223.8±2.6	246.3±10.4
	p-value		<0.001	<0.001
	*P**-value			0.1
Lead (mg/ kg)	Range	-	-	-
	Means±SD	Not detected	Not detected	Not detected
Cadmium (mg/ kg)	Range	-	-	-
	Means±SD	Not detected	Not detected	Not detected

-Data are represented as means ± SD of 3 farms.; P-value :- when different groups compared to EL-Galini farm.; P*-value :- when different groups compared to Locanda farm.; . p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation

Accumulation of some heavy metals in fish gills of three different farms

Table (7) obtains a significant increase of Zn and Pb in both Locanda and EL-Bats farms with a *P*-value of 0.01, 0.001 for Zn and 0.05, 0.05 for Pb, respectively when compared to EL-Galini farm and recorded a *P**-value of 0.005and 0.06 when compared to Locanda farm. But, a significant decrease of Fe was recorded in both Locanda and

EL-Bats farms with a *P*-value <0.001 and <0.001) respectively, when compared with EL-Galini farm and *P**-value (0.01) when compared with Locanda farm. Furthermore, a non-significant variation in levels of Cu was detected in both Locanda and EL-Bats farms when compared to EL-Galini farm, Cd recorded high levels in EL-Bats farm compared with EL-Galini and Locanda farms. All heavy metals under study showed variation in contamination in all three different farms in following order Fe > Zn> Cu> Pb> Cd.

Table 7. Accumulation of Heavy Metals in Fish Gills of Three Different Farm (mg/kg).

Heavy metals \ Groups		EL-Galini Farm	Locanda Farm	EL-Bats Farm
Zinc (mg/ kg)	Range	40.3-40.4	45.2-45.5	65.3-65.3
	Means±SD	40.35±0.07	45.35±0.2	65.30±0.0
	p-value		0.01	0.001
	*P**-value			0.005
Copper (mg/ kg)	Range	3.0-4.0	4.0-8.0	2.0-5.0
	Means±SD	3.40±0.5	5.90±2.4	3.50±2.4
	p-value		0.3	0.9
	*P**-value			0.4
Iron (mg/ kg)	Range	1837.6-1852.0	402-409.6	515.6-528
	Means±SD	1844.8±10.1	405.3±5.4	521.8±8.7
	p-value		0.0	<0.001
	*P**-value			0.01
Lead (mg/ kg)	Range	0.2-0.2	0.35-0.4	0.8-0.9
	Means±SD	0.2±0.0	0.36±0.0	0.85±0.07
	p-value		0.05	0.05
	*P**-value			0.06
Cadmium (mg/ kg)	Range	0.1-0.1	0.1-0.1	0.0-0.0
	Means±SD	0.1±0.0	0.1±0.0	0.04±0.0

-Data are represented as means ± SD of 3 farms.; P-value :- when different groups compared with EL-Galini farm.; P*-value :- when different groups compared with Locanda farm.; . p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation

Accumulation of heavy metals in fish skin of three different farms

Table (8) reveals that, there was a significant decrease of Zn and Fe with *P*-value (<0.001, 0.01 for Zn) and (0.001,< 0.001 for Fe) respectively in Locanda and EL-Bats farms when compared with EL-Galini farm and *P**-value (<0.001, 0.04) when compared with Locanda farm. In addition, there was non- significant variation of levels with mean value ± S.D of Cu in both Locanda and EL-Bats farms when compared to EL-Galini farm. While, neither Cd nor Pb was detected in skin organ of fish at the three different farms under study except in EL-Bats farm that recorded levels with mean value ± S.D (1.4±0.07). This was accompanied with the fact that fish were found with enlarged gallbladder compared to EL-Galini farm fish pop eyes (exophthalmia), darkening, loss of appetite, swollen gallbladder, brown/dark areas in the liver and a lot of fish died in this pond due to *streptococci bacteria*. All heavy metals under study showed variation in contamination in the three different farms in following order Fe> Zn> Cu.

Table 8. Accumulation of Heavy Metals in Fish Skin of Three Different Farms (mg/kg).

Heavy metals / Groups		EL-Galini Farm	Locanda Farm	EL-Bats Farm
Zinc (mg/ kg)	Range	143.6-144.7	90.2-90.9	133.5-134.1
	Means±SD	144.15±0.7	90.55±0.5	133.8±0.4
	p-value		<0.001	0.01
	*P**-value			<0.001
Copper (mg/ kg)	Range	2.36-5.50	1.10-4.90	3.80-6.70
	Means±SD	3.39±2.2	3.00±2.6	5.25±2.0
	p-value		0.7	0.6
	*P**-value			0.4
Iron (mg/ kg)	Range	3330-3330	279-293	402-402
	Means±SD	3330±0.00	285.6±9.8	401.8±0.2
	p-value		0.001	0.00
	*P**-value			0.04
Lead (mg/ kg)	Range	-	-	1.0-2.0
	Means±SD	Not detected	Not detected	1.4±0.07
Cadmium (mg/ kg)	Range	-	-	-
	Means±SD	Not detected	Not detected	Not detected

-Data are represented as means ± SD of 3 farms.; P-value :- when different groups compared to EL-Galini farm.; P*-value :- when different groups compared to Locanda farm.; p > 0.05 is non- significant - p ≤0.05 is significant - SD ± standard deviation

DISCUSSION

Heavy metals in the aquatic environment is a main problem due to its toxicity and danger to plant and animal life, that affect the natural ecological balance. Residue is the net build-up of substances from water in an aquatic organism that results from enhanced uptake and slow elimination of such substance **(Bhattacharya *et al* , 2008)**. The current study recorded that the values of heavy metals showed relative variation among three different drains. Results showed that, the order of heavy metals were Fe> Zn > Cu> Pb> Cd in all three different drains. Additionally, the values of heavy metals showed relative variation Fe> Zn> Cu> Pb> Cd in all three different farms that agrees with the result reported in the study of **Adham *et al.* (2002)** who studied contamination of heavy metals (Fe, Cu, Zn, Cd and others) at Maryut lake in difference stations. Moreover, the present finding coincides with that of **Ali and Abdel-Satar (2005)** who studied the heavy metals (Fe, Mn, Zn, Cu, Pb and Cd) in water of some fish farms in EL-Fayoum (Goda1,2; EL-shoura and shalakany). In addition, **Zaghloul (2008)**, **Abdel-Satar *et al*. (2010)** and **Gohar *et al*. (2018)** studied contamination of heavy metals , such as Fe, Zn ,Cu, Pb , Cd in water samples of El-Bats drainage canal and El-Wadi drainage canal. Moreover, **Emara *et al*. (2015)** detected accumulation of heavy metals of Fe, Zn, Cu, Pb, Cd at Al-Abbassa and Shader Azzam fish farms. It is worth mentioning that, **Abd El-Atti *et al*. (2018**) reported that, water collected from Qarun and Burullus Lakes had high levels of lead and cadmium. The concentration of Pb and Cd in Lake Qarun were much lower than that of Burullus Lake. As any pollutants in water has negative impact on survival of fish, water must be purified from those pollutants, especially heavy metals. Thus, the present study was concerned about treatment of drainage water which pour into fish farms by using simple and cheap treatment methods, such as treatment by pumpkin (*Cucurbita pepo*) and eggplant (*Solanum melongena).* Heavy metal concentrations in El- Bats drainage before and after treatment by pumpkin showed a relative variation among different heavy metals after treatment with pumpkin. It is clear from obtained results that, the removal of the different heavy metals follow the order of: Fe> Cd> Zn> Cu> Pb. The maximum removal was 77% for Fe, and the minimum was 50% for Pb. It is clear from obtained results that, the order of removal of the different heavy metals were arranged as follows: Fe> Cd> Cu> Zn and Pb (Zn and Pb had equal removal percentage). The maximum removal was 88% for Fe and the minimum was 25% for Zn and Pb under effect of treatment by eggplant. Compression between the two methods for heavy metals removal showed that, pumpkin was good for treating Zn and Pb the maximum removal for Zn was 65% and for Pb the value was 50%.While eggplant was good for treating Fe, Cd and Cu recording maximum values (88%, 78% and 77% respectively). The aforementioned results are similar to those of **Samuel *et al*. (2016)** who stated that, pumpkin was an excellent adsorbent for ameliorates of Cu(II) and Ni (II) ions. Additionally, **Deepthi *et al*. (2017)** reported that, the natural coagulants pumpkin seeds have the potential to remove copper from water, while **Karimi *et al*. (2018)** concluded that, treated eggplant peel used as an adsorbent for Pb from aqueous solution recorded good results for lead removal from wastewater. Analyses of serum biochemical constituents' levels have shown useful information in detection and diagnosis of metabolic orders and diseases in fishes **(Ferrari *et al*., 2007)**. The present study detected variation in values of biochemical parameter levels recording a significant increase in both Locanda and EL-Bats farms when compared to EL-Galini farm under study which indicated disrupted carbohydrates breakdown metabolism. The promote breakdown of liver and muscle glycogen via glycogenesis mediated may be due to adrenocortical and catecholamine hormones and also reduction of insulin secretion **(Gad, 1999)**. The current result concurs with that of **Adham *et al*. (2002)** who studied the level of glucose in the Nile tilapia at Maryut lake and **Osman *et al*. (2018)** who reported that, glucose in the blood serum of the Nile tilapia increased in Aswan, Kena, Assiut, Beni Suef, Damietta and Rosetta. In addition, the present study recorded a significant decrease of creatinine, CK and AST in both Locanda and EL-Bats farms when compared to EL-Galini farm in addition to urea in Locanda farm. However , a significant increase was detected in EL-Bats farm when compared to EL-Galini farm. Moreover , the present findings detected a significant increase of ALT in Locanda farm, while a significant decrease was spotted in EL-Bats farm when compared to EL-Galini farm. This result is similar to that of **Zaghloul (2008)** who reported that , there was a significant increase in serum AST and ALT of *Clarias gariepinus* collected from the agricultural drainage canals, El-Bats and El-Wadi drains. Addingly, **Osman *et al.* (2018)** stated that, ALT and AST increased in blood serum of the Nile tilapia in Aswan, Kena, Assiut, Beni Suef, Damietta and Rosetta. This increase in the studied enzymes activities may be attributed to the damage in the liver tissues, liver enzyme inhibition by the action of the recorded bio accumulated heavy metals disturbance in Kreb's cycle as reported by **Sanchez *et al*. (2005)**. The present study determined that, levels of CK showed an increase in the Nile tilapia of EL-Galini farm compared to others. This result agrees with that of **Adham *et al*. (2002)** who obtained level of CK in serum of the Nile tilapia at Maryut Lake and **Yousaf and Powell (2012)** who studied levels of CK in Atlantic salmon. ALP showed an increase in the Nile tilapia of EL-Bats farm compared to others; and agrees with the result reported by **Adham *et al*. (2002)** recording high increase in serum of the Nile tilapia fish at Maryut Lake. Additionally, **Dorcas and Solomon (2014)** and **Osman *et al.* (2018)** showed that there was variation of values of hormone parameter levels (T3, T4, TSH, GH and Cortisol) of the Nile tilapia in three different farms with significant increase in levels of all hormones under study in the blood of the Nile tilapia of Locanda farm compared to EL-Galini and EL-Bats farms, respectively. Consequently, the present results discussed residue of heavy metals of Fe, Cu, Zn, Cd and Pb in the blood of the Nile tilapia in three different farms. Moreover, it revealed that, there is a variation in values of heavy metals of the Nile tilapia fish blood and detectable in order of: Zn> Fe> Cu in fish of EL-Galini Farm and Locanda farm. While, the order was Zn> Cu> Fe in fish of EL-Bats farm and not detectable for Pb and Cd in Locanda and EL-Gilini fish farms, but detected in EL-Bats. Residue of heavy metals varied between species, ages, sex and organs; liver and gills have metabolic activity in which high levels of metals were found. Nevertheless, muscles showed low metabolic activity where less level of heavy metals were found (**Younis *et al.*, 2015**). Residue of heavy metals in different organs, such as liver, gills, muscles and skin discussed in the present study showed that, all heavy metals under study showed variation in residue in all three different farms. The order was Fe> Cu> Zn and Cd> Pb in the Nile tilapia fish liver organ, but in fish muscles the order was Fe> Zn> Cu with no detectable Pb or Cd. While, in gills, the order was Fe > Zn> Cu> Pb> Cd. On the other hand, the residue of heavy metals in fish skin of three different farm was in the following order: Fe> Zn> Cu, and no detectable Pb or Cd was spotted except in fish skin of EL-Bats Pb due to the existence of *streptococci* bacteria. This result is similar to those of **Yacoub *et al*. (2008)**, **Ghannam *et al.* (2014)** and **Talab *et al.* (2014)**. The previous authors found that, the order of heavy metals was Fe> Zn> Cu > Cd > Pb in liver, gills, muscles and intestine organs of *O. niloticus* (L.) living in fish farm in El Fayoum Province that coincides with the results of **Kumar *et al*. (2011)**, **Shivakumar *et al*. (2014)**, **Ghannam *et al*. (2015a, b)**, **Talab *et al*. (2016)** and **Abd El-Atti *et al.* (2018).**

CONCLUSION

The present study was concerned with the effect of different drains on the fish farms by the examination of the concentration of different heavy metals in water and fish collected from drains and farms. The Nile tilapia was used as a biomarker of pollution with heavy metals by detecting their accumulation in different fish organs and blood. Water treatment using low adsorbent (pumpkin and eggplant) to remove this heavy metals showed that, pumpkin was good for treating Zn and Pb ,while eggplant was good for treating Fe, Cd and Cu .Additionally, this work addressed the influence of heavy metals on fish biochemistry by detecting glucose, liver and kidney functions, cortisol hormone, growth and thyroid hormones. All the results emphasized that contamination with heavy metals such as zinc, iron, copper, lead and cadmium in water of drainage and fish farms is very hazardous to aquatic life and fish culture.

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