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Effect of Heavy Metals Contamination on Biochemical Parameters of the Nile Tilapia from Different Fish Farms

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ABSTRACT

This study is concerned with the contamination of heavy metals and its effect on biochemical parameters and enzyme activity of the Nile tilapia collected from three fish farm. For this reason the physical and chemical parameters of farms water were examined. Also, heavy metals (Zn, Cd, Pb, Fe, and Cu) were measured in water, fish blood, and sediment collected from the farms under study. The results of pH in the three farms EL-Galini, Locanda, and EL-Bats were 7.95, 8.03, and 8.06, respectively where, dissolved oxygen were 5.9, 7.2, and 6.0 (mg/l). The values of heavy metals in water showed relative variation Fe>Zn>Cu>Pb>Cd in all three different farms. For sediment EL Galini farm have the highest value for all the examined heavy metals followed by Locanda and EL-Bats farm. The results showed a significant increase in levels of (Random blood sugar), and (Alkaline Phosphatase) with *P*-value 0.004, 0.002 respectively in both Locanda and EL-Bats farms when compared with EL Galini farm. Also, there was a significant decrease in levels of creatinine, (Creatinine Kinase) and (Aspartate Transferase) with P-value 0.02 and 0.05 for creatinine, <0.001 and <0.001 for CK and <0.001 and <0.001 for AST, respectively in both Locanda and EL-Bats farms when compared with EL Galini farm.

INTRODUCTION

Water is very important for fish and its quality is one of the main factor in pond management until it affects fish production. The quality of water generally means the water parameter which must be present for optimum growth of aquatic organisms (**Ehiagbonare and Ogundiran, 2010**). Water quality is made up of physical, chemical and biological factors which effect on the use of water for fish farming purposes. These factors include dissolved oxygen, pH, temperature, and electrical conductivity. Aquaculture, a rapidly expanding business in the Asia-Pacific

since 90s, has been an important source of food security and employment in many countries **(Stanley, *et al.*, 2003**). Tilapia (*O. niloticus*) is a widely-cultured species in the ponds of the country, fish productivity depends on the physicochemical characteristics of the water body. The environmental impact of aquaculture is observed in many ways including user conflicts, change of ecosystems and water pollution, of these possible negative impacts, water pollution of water resources is the most common complaint and has attracted the greatest attention through the nations (**Cripps and Bergheim, 2000**). The quality of water of any aquatic ecosystem arises by the interaction of physical chemical and biological component of the habitat. Aquatic biota presents in any aquatic ecosystem directly influence the physicochemical characteristics of that aquatic ecosystem. The physicochemical parameters have important significance in determining the trophic status of aquatic habitats (**Sharma, *et al.*, 2009**). Between aquatic organisms, fish cannot escape from the toxic effects of these pollutants, and are generally considered to be the most organisms for pollution monitoring in aquatic ecosystems (**Beyer and Vermeulen**, **2003**). So, fish samples can be considered as one of the most important indicators in freshwater systems for the respect of metal pollution rate (**Rashed, 2001).** The commercial and edible species have been studied in order to check for those harmful to human health, while blood is a path physiological reverse of the all body parts, and therefore, blood parameters are important in investigation the structural and functional state of fish insecure to toxicants (**Begüm, *et al.*, 2005).** Changes in the biochemical blood parameters indicate changes in metabolism and biochemical processes of the organism, resulting from the impacts of various pollutants, and they make it possible to study the mechanisms of the impacts of these pollutants (**Adhikari, *et al.*, 2004).**

So the aim of this work was the detection of different heavy metals in water, fish blood, and sediment collected from different farms (EL Gilani, Locanda and EL Bats farms) at El-Fayoum Government. Also the study investigate the effect of heavy metals on biochemical parameters and some enzyme activity offish collected from the three farms under study.

MATERIALS AND METHODS

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

Collection and analysis of Water and Fish samples

Water samples were collected from three different commercial farms (EL Gilani, Locanda and EL Bats farms, respectively). The physical and chemical parameters and heavy metals in water samples were determined according to **APHA (1998).** Sediment samples were dried at 80^0C in oven and grinding to fine particles, 1.0 g of fine grinded samples was digested according to **Kouadia and Trefry (1987)** method. Heavy metal

determination (Fe, Pb, Zn, Cu and Cd) in water and sediment samples were detected using atomic absorption (Perkin Elmer 3110 USA) with graphite atomizerHGA-600.

Fish samples Nile tilapia (*Oreochromis niloticus*) were collected from the same three commercial farms with weight (170-230 g) and length (20- 25 cm). Heavy metals in fish blood were detected according to **Barley and She** (**2013**) by inductively coupled plasma mass spectrometry (ICP-MS) at Soil and Water Laboratory, Faculty of Agriculture, Fayoum University.

Collection and analysis of blood samples

Blood samples were collected in eppendorf tubes from the caudal vein of *Oreochromis niloticus* then collected samples centrifuged at 3000 rpm for 15 minutes and the supernatant serum obtained by using micropipette and stored at 4˚C till determination of urea, creatinine, AST (Aspartate Transferase), ALT (Alanine Trasferase), (RBS) Random Blood Sugar, creatinine kinase (CK), alkaline phosphatase (ALP), and heavy metals (cadmium, zinc, iron, lead and copper). Urea, creatinine, ALT and AST level was carried out by a kit supplied by BioSystems, (Barcelona, Spain). (RBS) Random Blood Sugar was recorded according to **Trinder (1969)** using kit purchased from (Randox Laboratories, UK). ALP was carried out according to **Tietz, *et al*., (1983).** CK was carried out by UV kinetic method.

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)**.

RESULTS AND DISCUSSION

Physical and chemical parameters of waters from three different farms

The results of physical parameters in the three different farms water recorded in Table (1). The data recorded that there was non-significant variation at the level of pH and temperature of Locanda and EL-Bats farms when compared with EL-Galini farm. But, there was a significant decrease of total dissolved solids in Locanda and EL-Bats farms with *P*-value (0.002 and 0.005, respectively) when compared with EL-Galini farm and *P**-value (0.006) when compared with locanda farm.

Water temperature influences the onset of fish spawn, aquatic vegetation growth, and the biological demand for oxygen in ponds. The increases in temperature lead to decrease in oxygen, which give harmful effect on fish life and in some cases lead to fish death (**Ghannam, 2021).** Fish can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 (**Stevens**, **2009**).

Table (1). Physical parameters of water collected from three different fish farms.

Parameters	Groups	EL-Bats Farm	Locanda Farm	EL-Galini Farm
pH	Range	7.8-8.1	7.9-8.1	8.0-8.2
	Means±SD	7.95±0.22	8.03±0.18	8.06±0.85
	P-value		0.76	0.62
	P*-value			0.83
Temperature (°C)	Range	31-33	31–33	31-32
	Means±SD	32±1.4	32±0.70	31±2.1
	P-value		0.7	0.8
	P*-value			0.6
TDS (mg/l)	Range	47.0-47.12	11.0-11.21	11.2-11.7
	Means±SD	47.06±0.08	11.20±0.28	11.36±0.51
	P-value		0.002	0.005
	P*-value			0.06

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

The present study showed that the mean of pH in the three farms EL-Galini, Locanda, and EL-Bats were 7.95, 8.03, and 8.06, respectively; this indicates that the water of the three farms are suitable for fish. Chemical parameters at Table (2) recorded that, the results of ammonia, ammonia un-ionized nitrate, nitrite, and dissolved oxygen were non-significant in Locanda and EL-Bats farms when compared with EL-Galini farm.

But there was a significant decrease of levels of salinity and organic matter in Locanda and EL-Bats farms with P-value 0.01 and 0.02, respectively, when compared with EL-Galini farm, *P**-value (0.01) when compared with locanda farm. Also, our results detected significant increase levels of total alkalinity in Locanda farm and non-significant variation in EL-Bats farm when compared with EL-Galini farm.

Nitrogen compounds have been known as major metabolic products in fish farming, where nitrite may reach toxic concentrations in high density aquaculture systems and in flowing waters due to industrial pollution and agricultural wastes like fertilizer (**Collins, 1975**). Also, our results detected a significant increase of total alkalinity in Locanda farm when compared with EL-Galini farm however, it was detected non-significant variation in EL-Bats farm when compared with EL-Galini farm. **Abubakar (2012)** and **Coldebella *et al*. (2017)** recorded that alkalinity ranged 24.8 mg/l to 48 mg/l was suitable for fresh water fish culture. Salinity detected a significant decrease of levels of salinity in Locanda and EL-Bats farms with *P*-value (0.01) when compared with EL-Galini farm. **Zaghloul (2008)** showed deterioration in water quality of samples collected from the studied drainage canals (El-Bats and El-Wadi). Our study concerned with determination of organic matter in farms water in Locanda and EL-Bats farms with *P*-value (0.02) when compared with EL-Galini farm addition to dissolved oxygen value was observed non-significant in Locanda and EL-Bats farms when compared with EL-Galini farm. The results of dissolved oxygen in the three fish farms under study were 5.9, 7.2, and 6.0 for EL-Galini, Locanda, and EL-Bats farms, respectively. **Talab *et al*. (2016)** found that

there are many parameter effect on dissolved oxygen content like temperature, pH, and photosynthesis activity.

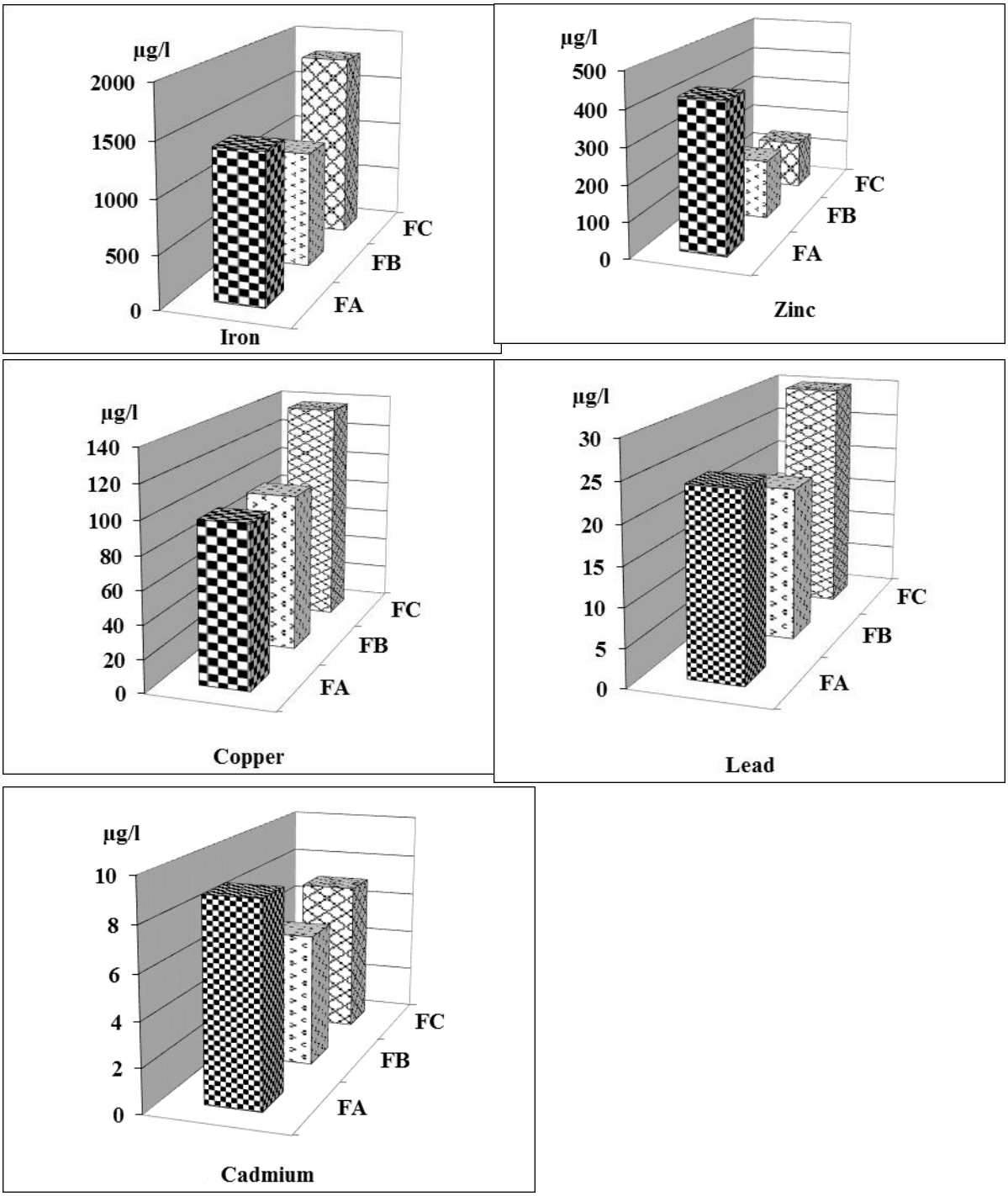
Table (2). Chemical parameters in water collected from three different fish farms.

Parameters	Groups	EL-Bats Farm	Locanda Farm	EL-Galini Farm
Ammonia (mg/l)	Range Means±SD *P*-value *P**-value	0.357-0.425 0.391±0.05	0.256-0.325 0.290±0.05 0.10	0.551-0.671 0.611±0.08 0.1 0.07
Ammonia Un-ionized (mg/l)	Range Means±SD *P*-value *P**-value	0.034-0.046 0.04±0.01	0.043-0.063 0.053±0.01 0.4	0.06-0.082 0.200±0.02 0.3 0.5
Total Alkalinity(mg/l)	Range Means±SD *P*-value *P**-value	280-300 90±14.1	140-145 145±7.0 0.02	280-300 294±14.1 1.00 0.02
Salinity (g/l)	Range Means±SD *P*-value *P**-value	8-9 8.5±0.7	1-1 0.77±0.3 0.01	1-1 0.75±0.3 0.02 1.00
Organic matter (mg/l)	Range Means±SD *P*-value *P**-value	21.00-21.36 21.18±0.3	7.32-7.6 7.33±0.4 0.003	3.0-3.72 3.38±0.5 0.003 0.02
Nitrate (mg/l)	Range Means±SD *P*-value *P**-value	0.122-0.158 0.141±0.02	0.105-0.116 0.110±0.01 0.3	0.182-0.218 0.200±0.03 0.1 0.1
Nitrite (mg/l)	Range Means±SD *P*-value *P**-value	4.211-5.312 4.766±0.76	5.024-6.214 5.619±0.84 0.4	8.205-9.306 8.755±0.78 0.04 0.06
Dissolved oxygen(mg/l)	Range Means±SD *P*-value *P**-value	5.6-6.2 5.9±0.4	6.7-7.8 7.2±0.7 0.2	5.7-6.3 6.0±0.4 0.8 0.2

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

Heavy metals contaminated in water from three different fish farms

As shown in Fig. (1), there is a significant decrease of Zn and Fe in Locanda and EL-Bats farms with *P*-value 0.001 and 0.001 for Zn and <0.001 and <0.001 for Fe, respectively, when compared with EL Galini farm and *P**- value was <0.001 and 0.01 when compared with Locanda farm. But, there was a significant increase of Cu in EL-Bats farms with *P*-value 0.004 and *P**- value 0.001 when compared with Locanda farm, and it was recorded non-significant variation in Locanda farm when compared with EL Galini farm. On other hand, there was non-significant change of Cd and Pb in both Locanda and EL-Bats when compared with EL Galini farm.



FA:-El- Gilani farm **FB:-**Locanda farm **FC:-** EL-Bats farm

Fig (1). Heavy metal concentrations (µg/l) in water collected from three different fish farms

The obtained results for heavy metals in water showed relative variation Fe>Zn> Cu>Pb>Cd in all three different farms. **Ali and AbdEl-satar (2005)** studied the distribution patterns of heavy metals (Fe, Mn, Zn, Cu, Pb, and Cd) for water in some fish

farms in EL-Fayoum (Goda1, 2; EL-Shoura and Shalakany). The allowed concentration of Fe, Pb, Mn, and Cu were 0.3, 0.01, 0.1, and 2.0 mg/L, respectively according to **WHO, (2011)** and **Egyptian Drinking Water Quality Standards (2007).** The results of all metals in the three farms did not exceed the allowed limits. The higher concentrations of Mn may be related to the release of manganese from dead aquatic plants that become easy due to the decrease in water level with settling in water current beside the dissolution of sediment manganese and its presence water **(Ghannam, *et al*., 2014).** The increase in Cu values may be related to the high evaporation rate and elevation in temperatures of air and water or the release of copper from sediment to surrounding water **(Warren and Zimmerman, 1994).**

Heavy metals contaminated in sediment from three different fish farms

The results of accumulation of different heavy metals in sediment of the three fish farms were found at Table (3). There were a significant decrease in levels of Zn, Cu, and Fe in both Locanda and EL-Bats farms with *P*-value 0.004 and 0.002 for Zn, 0.005 and 0.006 for Cu, and 0.001 and <0.001 for Fe, respectively, when compared with EL Galini farm, and *P**-value was 0.009, 0.01, and 0.002 when compared with Locanda farm. Also, Pb recorded low value in both Locanda and EL-Bats farms when compared with EL Galini farm, but Cd detected high value in Locanda farm when compared with EL Galini farm and EL-Bats farm.

Table (3). Accumulation of some heavy metals in sediment of three different farms

Heavy metals	Groups	EL-Galini Farm	Locanda Farm	EL-Bats Farm
Zinc (mg/kg)	Range	82.48-83.96	75.4-76.12	52.0-52.08
	Means±SD	83.2±1.0	75.7±0.5	52.04±0.05
	p-value		0.004	0.002
	*P**-value			0.009
Copper (mg/kg)	Range	33.84-43.56	20.56-28.76	7.56-11.68
	Means±SD	38.7±6.8	24.6±5.7	9.6±2.9
	p-value		0.1	0.01
	*P**-value			0.01
Iron (mg/kg)	Range	45040-45080	36760-36920	12249-12276
	Means±SD	45060±28.3	36840±113.3	12262.5±19.0
	p-value		0.03	<0.001
	*P**-value			0.002
Lead(mg/kg)	Range	7.0-7.0	6.0-6.0	6.0-6.0
	Means±SD	7.0±0.0	6.0±0.0	5.1±0.0
Cadmium (mg/kg)	Range	0.12-0.12	0.16-0.16	0.04-0.04
	Means±SD	0.12±0.0	0.16±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

It is clear from Table (3) that EL Galini farm have the highest value for all the examined heavy metals followed by Locanda farm and the lowest value were recorded in

EL-Bats farm. The presence of organic matter in sediments is a source of nutrients for the living fauna and consider as important factor in the accumulation and release of pollutants in the water (**Ahmed and Elaa, 2003).** The high values of organic matter in the sediments might be related to the flourishing of phyto and zooplankton, which lead to high organic productivity (**Boyd and Tucker, 1979).**

Accumulation of heavy metals in fish blood of three different farms

The concentration of different heavy metals in blood of Nile tilapia fish from different fish farm were found in Table (4). The order of metal distribution in EL-Galini farm was Zn>Fe>Cu and the same order was obtained in Locanda farm, while in EL-Bats farms the order was Zn> Cu > Fe. But, neither Cd nor Pb was detected in fish blood at the three different farms under study.

Zinc is an essential heavy metal for living organisms where the cells contain Zn as one of the essential components of various enzymes. It is involved in different aspects of cellular metabolism (**Osredkar and Sustar, 2011)**. It is also necessary for a healthy immune system, cell division, synthesis of protein and collagen etc. (**Yirgu, 2011)**. However, a higher amount of Zn have a toxic effect on human health (**WHO, 2011**). Copper is also an essential heavy metal and an important constituent of a living organism. It plays an important role in the production of hemoglobin, myelin, melanin, and it also helps in the normal functioning of the thyroid gland. As this mineral is present in many functions of the body, copper deficiency can produce an extensive range of symptoms like hernias, aneurysms, blood vessel breakage manifesting as bruising or nose bleeds (**Yirgu, 2011).**

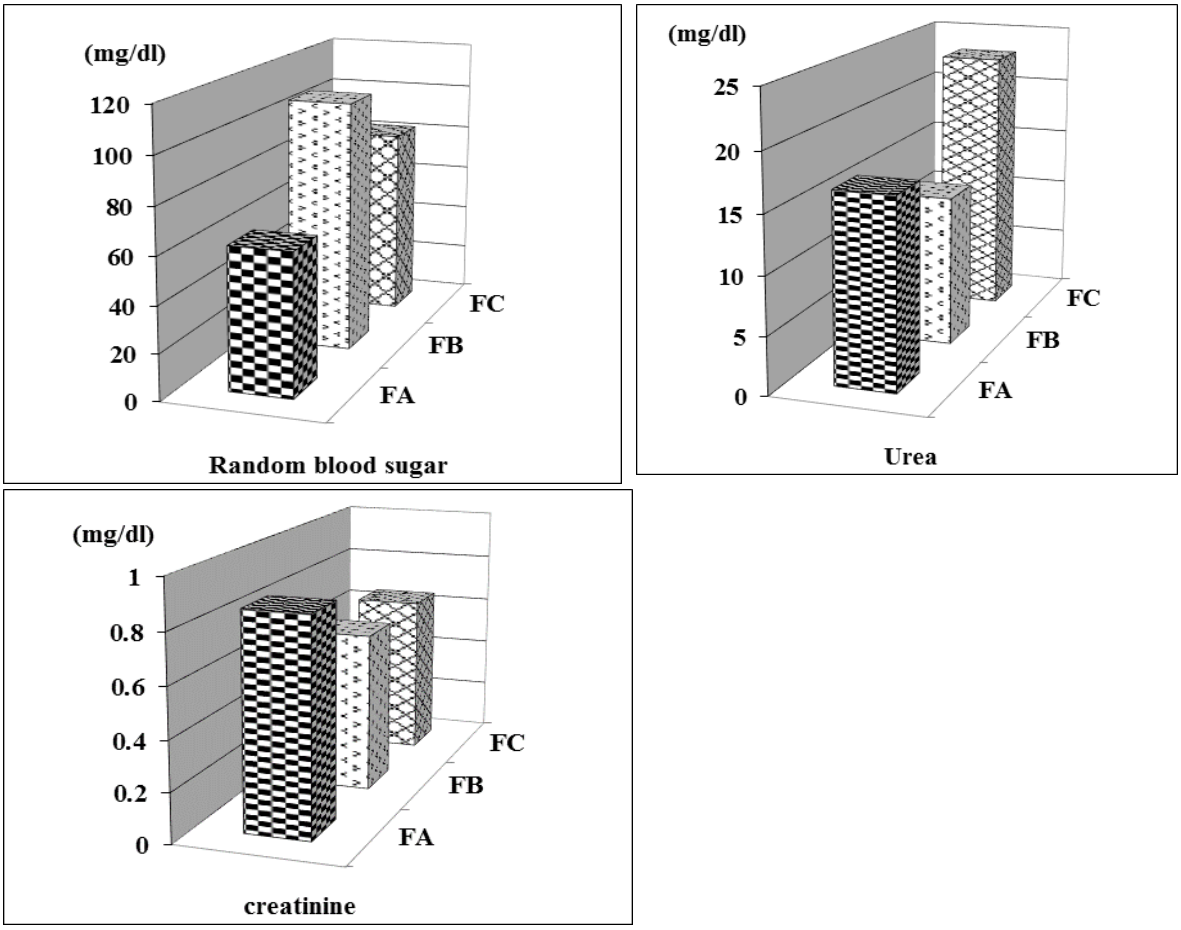
Table (4). Heavy metals concentrations of Nile Tilapia fish blood in three different farms:-

Heavy metals	Groups	EL-Galini Farm	Locanda Farm	EL-Bats Farm
Zinc (mg/dl)	Range Means±SD *p*-value *P**-value	365.0-365.9 3365.45±0.64	562.7-563.1 562.90±0.28 <0.001	514.7-515.0 514.89±0.16 0.001 <0.001
Copper (mg/dl)	Range Means±SD *p*-value *P**-value	104.5-105 104.75±0.35	90-90.2 90.10±0.14 0.01	159-159.9 159.45±0.64 0.001 0.003
Iron (mg/dl)	Range Means±SD *p*-value *P**-value	201-203 203.00±2.83	233.5-234 233.80±0.42 0.04	157-157.6 157.30±0.42 0.001 <0.001

-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

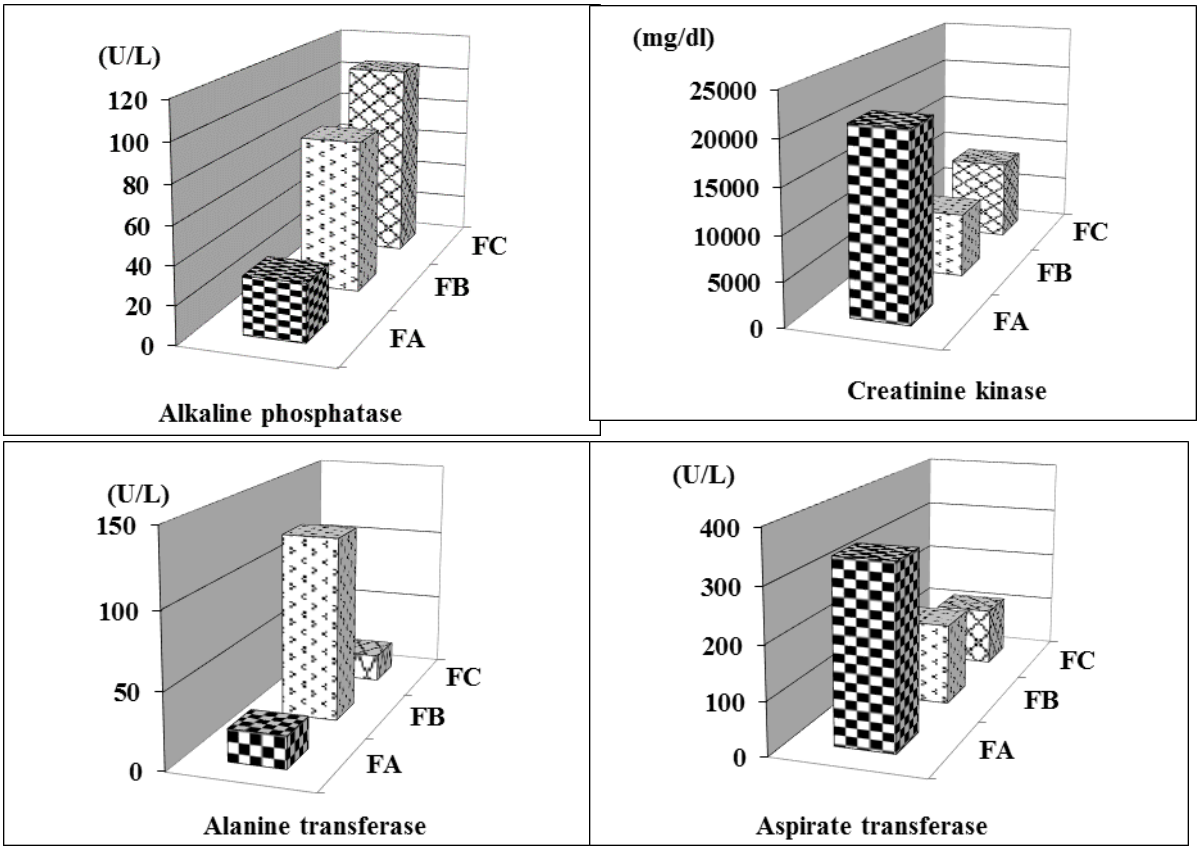
Biochemical and enzyme parameters in blood of Nile tilapia fish

Figs. (2, 3) show a significant increase of levels of RBS (Random blood sugar) and ALP (Alkaline Phosphatase)with *P*-value 0.004 and 0.002 for RBS (Random blood sugar) and 0.01 and 0.001 for ALP, respectively in both Locanda and EL-Bats farms when compared with EL Galini farm, and *P**-value was 0.002 and 0.001 when compared with Locanda farm. Also, there was a significant decrease in levels of creatinine, CK (Creatinine Kinase) and AST (Aspartate Transferase) with P-value 0.02 and 0.05 for creatinine, <0.001 and <0.001 for CK and <0.001 and <0.001 for AST, respectively in both Locanda and EL-Bats farms when compared with EL Galini farm; *P**-value was <0.001 when compared with Locanda farm. On other hand, there was a significant decrease in levels of urea in Locanda farm (*P*-value: <0.001), but a significant increase (*P*-value: 0.04) in EL-Bats farm when compared with EL Galini farm, and *P**-value was 0.03 when compared with Locanda farm. Our results detected a significant increase of ALT in Locanda farm (*P*-value: 0.01), but a significant decrease (*P*-value: 0.01) in EL-Bats farm when compared with EL Galini farm, and *P**-value was 0.002 when compared with Locanda farm. .



FA:-El- Gilani farm. **FB:**-locanda farm **FC:**-EL-Bats farm

Fig. (2). Biochemical levels in blood of Nile tilapia fish from three different fish farms.



FA:-El- Gilani farm. **FB:**-locanda farm **FC:**-EL-Bats farm

Fig. (3). Enzymes level in blood of Nile tilapia fish from three different fish farms.

Variation in values of biochemical parameters levels at Fig. (2) indicated disrupted carbohydrates breakdown metabolism that result from the increase in Random blood sugar. This may be due to promote breakdown of liver and muscle glycogen via glycogenesis mediated perhaps by adrenocortical and catecholamine hormones and also reduction of insulin secretion, **Gad (1999).** The 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 and pesticides and/or disturbance in Kreb's cycle as reported by **Sanchez *et al.*,** (**2005**).

CONCLUSION

The present study concerned with the effect of residual pollutant on the fish farms by the examination of the concentration of different heavy metals in water, sediment, and fish blood collected from three different fish farms. The Nile tilapia was used as a biomarker of pollution with heavy metals by detecting their accumulation in blood. This work studies the influence of heavy metal on fish biochemistry by detecting glucose, liver and kidney functions and also the effect of metals on some blood enzyme activity. All the

results emphasis that contamination of heavy metals such as zinc, iron, copper, lead, and cadmium in water fish farms is very hazards to aquatic life and for fish culture.

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