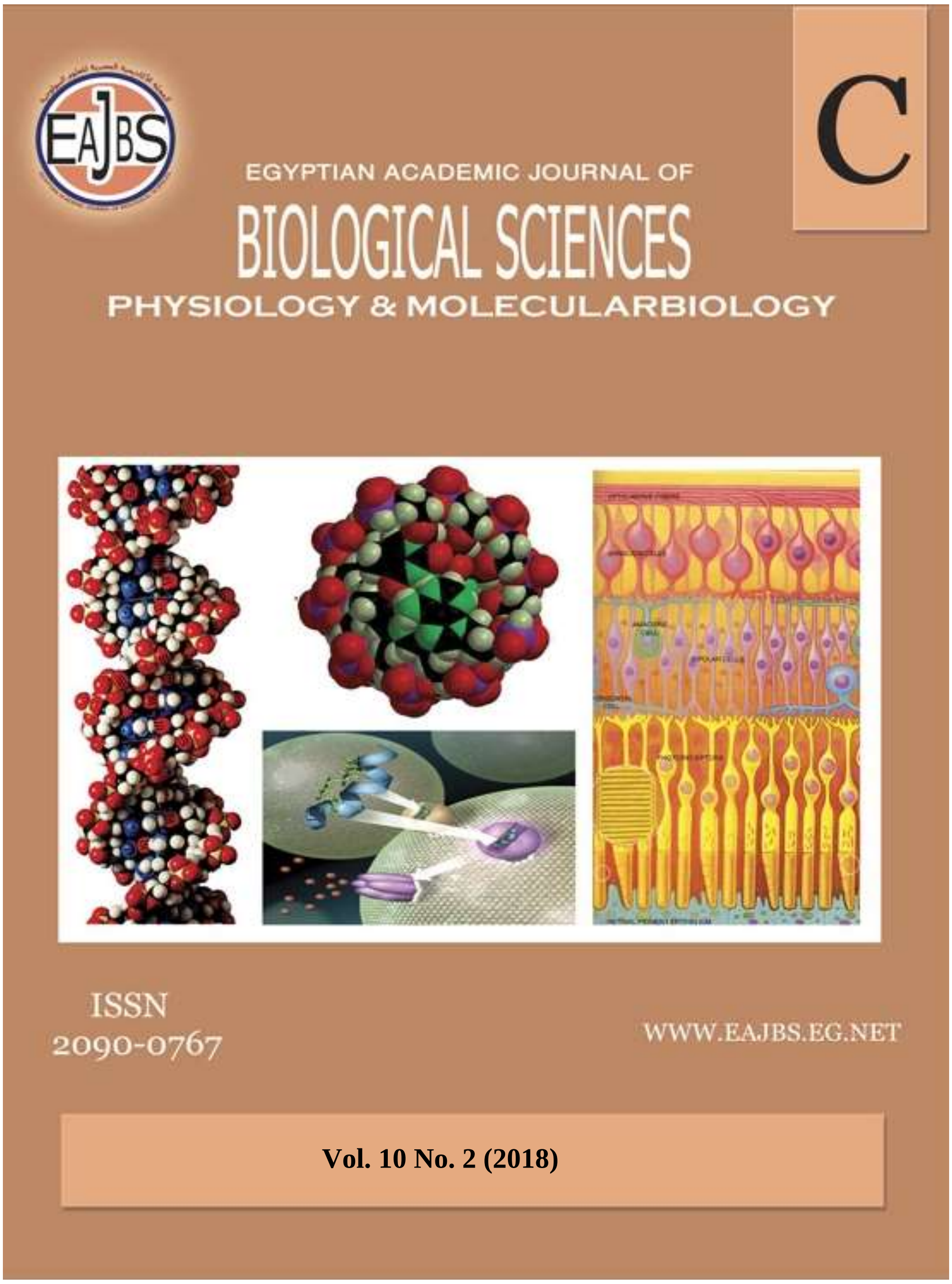

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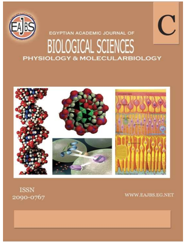

Assessment of some Heavy Metals in Water and Tissues of Tilapia Spp. Collected from Wadi El-Rayan Lakes. El-Fayoum Egypt and their Impacts on some Biochemical Parameters.

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ABSTRACT

The present study was carried on the Wadi El - Rayan lakes from spring 2015 to winter 2016. Seasonal water samples were collected from several selected sites to cover most parts of the two lakes to assess some heavy metals concentration (Fe, Pb, Cu, Cd, Zn and Mn). Samples of Tilapia Spp. fish were collected regularly during four successive seasons from Wadi El-Rayan Lakes (upper) and (lower). To evaluate the concentration of different heavy metals in the tissues (muscles, liver, gills). Also Biochemical parameters in fish serum such as analysis of (Glucose, Urea, Creatinine, the activity of liver enzymes (AST - ALT) enzyme activity, Triglycerides, Total Cholesterol, and Total proteins were analyzed. The results showed that the level of heavy metals (Fe, Pb, Cu, Cd, Zn and Mn) in the permissible limit except (Fe and Pb) were higher than the permissible limit and it concentration in water samples follow the order Fe > Mn> Pb> Cu> Zn and Cd also, the results showed the concentration in fish (muscles) within the permissible limits except (Fe). The results of biochemical parameters showed an increase in these parameters (serum Glucose, Urea, Creatinine, Cholesterol, Triglycerides, Total proteins, AST and ALT), this increase was significant in (Glucose, Urea, Creatinine, Cholesterol, Triglycerides, AST and ALT activities) and not significant in (Total proteins).

INTRODUCTION

Water pollution is a serious environmental problem in the world. It is the degradation of the quality of water that reduces water unsuitable for its intended purpose. Anything which degrades the quality of water is termed as a pollutant. Water pollutants can be broadly classified as major categories namely organic, inorganic, suspended solids and sediments, heavy metals, radioactive materials and heat (Botkin and Keller, 2008).

Wadi El-Rayan is a great depression (703 km^2) situated in the Western Desert, 40 km southwest of El-Fayoum Province. Wadi El Rayan Lakes is one of the most recent man-made lakes. Since 1973, the depression has been used as a water reservoir for agricultural drainage water exceeding the capacity of Lake Qarun.

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Wadi El-Rayan depression was connected to the agricultural drainage system of El-Fayoum Governorate- through El-Wadi Drain- in an effort to decrease the accumulation of excess drainage water in Lake Qarun and to protect the nearby agricultural land from inundation. Nowadays, it holds two main lakes, at different elevations, connected by a swampy channel. The first lake of Wadi El-Rayan covers an area of about 55.9km² at 10 m below the sea level and receives frequent effluent of wastewater from El-Wadi Drain (about 200 million cubic meters of agricultural drainage water are transported annually)(El-Shabrawy, 2007).

Now, the main threat to the effective long-term protection of the Wadi El-Rayan protected area is seen to be the development of un-controlled economic activities within its boundaries. These activities include large-scale land reclamation schemes, major oil extraction operations, rapidly expanding aquaculture, commercial fishing, and tourism, as well as the human settlement in highly sensitive areas such as previously un-disturbed habitats used by gazelles and other key species (IUCN, 1998a & b). furthermore, the extremely high rate of evaporation coupled with very low precipitation, both characteristic of arid areas, and the fact that the lakes are being formed from agricultural drainage wastewater could lead to increased concentration of pollutants and a rapid increase in salinity of the lakes (Saleh *et al*., 1988), where pollutants from agricultural waste including pesticides and fertilizers as well as other effluents of industrial activities and runoffs certainly will pass into the biotic elements of the ecosystem (Sayed and Abdel-Satar, 2009).

Wadi EI-Rayan lakes receive the agricultural wastewater drainage from El-Wadi Drain and vary in their physical and chemical characters, which controlling its area and volume (Mohamed and Gad, 2008).

Fish are considered one of the most susceptible aquatic organisms to toxic substances present in water (Alibabić *et al*., 2007), and at the same time considered as the major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health therefore, various studies have been taken worldwide on the contamination of different fish species by heavy metals (Sivaperumal *et al.*, 2007., Bhattacharya *et al*., 2010., and Gad, 2010)

Wadi EI-Rayan attracts the attention of many authors because of its historical and scientifically important to study its unique ecosystem, the study deals with water quality of the lakes (Sayed and Abd El-Satar, 2009) macrophyte and aquatic communities (El-Shabrawy, 2007).

The current study aimed To determine the accumulated level of some heavy metals (Fe, Pb, Cu, Cd, Zn, and Mn) in water and fish (Tilapia Spp.) tissues (muscles, liver, and gills). And To determine the change in some biochemical parameter in fish serum (Glucose, Urea, Creatinine, Total Cholesterol, Triglyceride, Total Protein, AST and ALT enzymes activities).

MATERIAL AND METHOD

Area of Investigation:

Wadi El-Rayan Depression has an area of 703 km² with two successive lakes connected through a channel. The total area of the two lakes is about 113.85 km² represented: The 1st lake (upper lake). Total area is 55.9km² , with a total perimeter 47.28km, the maximum width: 10.37km and the maximum length 6.610km, the maximum depths of the first lake is 23m, this lake takes a circular shape and receives frequent effluent of wastewater from El-Wadi Drain (about 200million cubic meters of agricultural drainage water are transported annually) The 2nd lake (lower lake).

Total Area is 57.95km^2, total perimeter 63.81 km the maximum width: 4.498km, the maximum length15.72km, the maximum depths of the second lake is 33m, this lake takes a conical shape. It is changing all the time, where newly flooded areas are continuously added at the southwestern side of the lake. The inflow water to the second lake varied from 3.66 × 106 m^3 in July to 17.68 × 106 m^3 in March with a total annual of 127.2 × 106 m^3 /year. The connecting channel: about 5Km long and vertical drop of 2.5m is characterized by permanent shallow water providing suitable habitat for a continuous cover by emergent aquatic macrophytes thus leading to a swamp formation (El-Shabrawy, 2007).

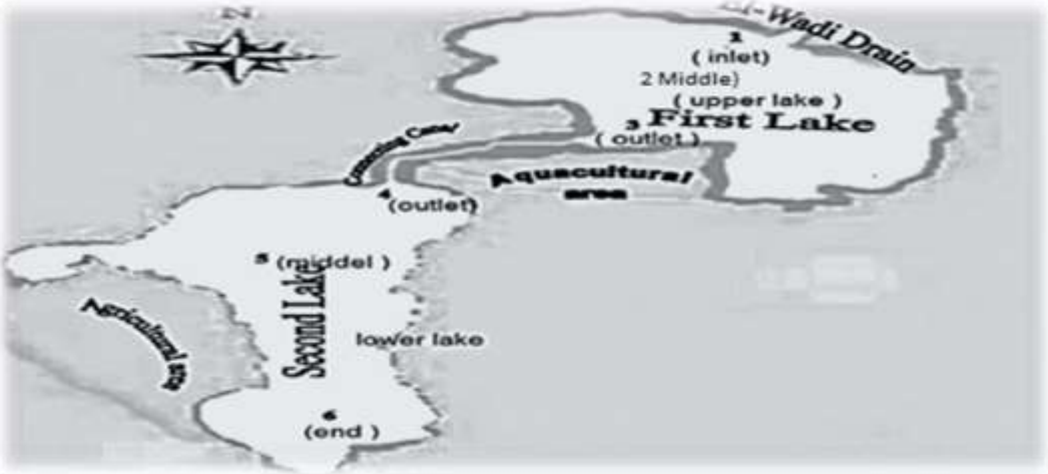

Fig. 1: Map of Wadi El-Rayan Lakes showing the selected sites.

Sampling Sites:

Six sites were selected for water and Tilapia Spp. fish samples were collected from Lake (1) and lake (2) from spring 2015 to winter.

Water Sampling:

Water samples were collected seasonally with one-meter depth from six sites which represent hotspot sectors in Wadi El-Rayan lakes Water samples were stored at 4°C and transported to the laboratory for heavy metals "Iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb) and cadmium (Cd) "analysis water samples were analyzed according to the standard methods for the examination of water and waste water (APHA, 2012).

Fish Sampling:

Tilapia Spp. fish samples were collected from six sites as showed in Fig(1) and Table (1) and control fish collected from un polluted fish farms . Fish were collected seasonally from each sampling site, with an average body weight (150gm ±20gm) and average body length (20±4 cm) upon collection. Fish collected from the selected sites were externally dried from water , and blood samples were collected immediately from fresh a live fish, blood samples were withdrawn from the arterial caudalis, the needle is run quite deep as much as possible through the middle line just behind the anal fin in a dorso-cranial direction by drawing the needle gently backward, then Blood was left to clot then centrifuged at 3000 r.p.m for 10 minutes .supernatant serum was obtained by using micropipette and stored at 4°C. Till determination of (Creatinine, blood urea, Total cholesterol, triglycerides, Total proteins, AST and ALT) and determine glucose immediately.

Table 1: The Latitude and Longitude of the sampling sites at Wadi El-Rayan lakes.

Lakes	Sites	Features of sites	Latitude	Longitude
First lake (upper)	1	In front of EL-Wadi drain	29° 15' 27.03"	30° 34' 44.89"
	2	Middle the upper lakes	29° 14' 35.79"	30° 29' 18.01"
	3	In front of the connecting canal	29° 14' 10.05"	30° 27' 17.93"
Second lake (lower)	4	In front of the entrance of water to the lower lake	29° 12' 33.55"	30° 25' 11.40"
	5	Middle of the lower lake	29° 10' 58.33"	30° 24' 16.87"
	6	Southern of the lower lake	29° 9' 2.42"	30° 24' 29.56"

Water Analysis:

Six elements included Fe, Pb, Cu, Cd, Zn and Mn, were measured in water samples collected seasonally from the investigated area. The metals concentrations were determined after the digestion by nitric acid as described in APHA (2012), and measured by using atomic absorption spectrophotometer (GBC atomic absorption spectrophotometer Savanta AA) was used for measured portions of digested solutions.

Fish Analysis:

For Biochemical analyses Plasma glucose was determined using the method of (Burtis *et al.,* 1996) (Spinreact Diagnostics kit). Plasma total protein by using the method of (Josephon, 1957).Plasma creatinine was estimated by to the method of (Murray, 1984) (Diamond Kits) .Urea was determined according to the method of (Fawcett and Scott, 1960) (Diamond kits).Plasma total cholesterol according to the method of (Richmond, 1973) (Human kits). Triglycerides were determined using the method of (Schettler, 1975) (Human Kits). The activities of plasma AST and ALT were estimated according to the method of (Schumann *et al*., 2002) (Human kits). For heavy metal analysis Samples of Tilapia spp. were collected from Wadi El-Rayan Lakes (1) and (2). Some specimen of dorsal lateral muscle, liver, and gills of Tilapia spp. were dissected cut and kept frozen at 20ºc for heavy metal analysis. Muscles, Liver, and Gill from six individuals of fish were mixed together forming a composition. For heavy metal analysis 15gm from each composed mixture were dried for 24 hours and then grounded to a fine powder. The dried tissues were digested as following: 1.0 g of dried powder was digested in 5ml nitric acid and 5 ml perchloric acid, in a beaker boiled on the hot plate at 80-90 °C until the sample becomes clear. After cooling, the solution was filtrated and transferred to 50 ml volumetric flask and fill up to the level with de-ionized water. The digests were kept in plastic bottles and later; The levels of Fe, Pb, Cu, Cd, Zn, and Mn in digests were determined using atomic absorption spectrophotometer (GBC atomic absorption spectrophotometer Savanta AA). The results were expressed in (µg/g dry weight).

Statistical Analyses:

The experimental data were subjected to Statistical analysis by using one-way analysis of variance (ANOVA). The difference checked by one way (ANOVA) was significant when ($p \leq 0.05$) and highly significant when ($p \leq 0.01$). Each reading of biochemical parameters represents (Mean ± standard error) of 7 fish.

RESULTS AND DISCUSSION

Heavy metals in water can be partitioned into dissolved and suspended fraction. It is well known that most dissolved heavy metals are present as organic complexes in natural water (Prego and Cobelo-Garcia, 2003). The concentration of selected metals are listed in Table (2).

Table 2: Heavy metals concentrations (µg/l) in water collected from Wadi El-Rayan Lakes

Metal ions **(µg/L)**	Lakes	Sites	Spring	Summer	autumn	winter	Mean ±S.E	Egyptian Environmental low 48 decision 92/2013
Fe	**1**	1	340.0	572.4	409.8	424.8	436.7±40.03	<500
		2	324.2	445.2	445.8	1603.6	704.6±246.7	
		3	381.0	422.0	448.2	1430.2	670.3±207.78	
	2	4	220.4	380.3	588.6	775.8	491.2±99.31	
		5	143.0	345.0	459.6	427.8	343.8±58.31	
		6	161.0	250.0	443.8	386.4	310.3±52.45	
Pb	**1**	1	64.8	82.48	18.58	48.26	53.5 ± 11.13	<50
		2	58.0	77.8	21.68	41.94	49.8 ±9.76	
		3	73.0	65.0	39.27	47.2	56.1 ±6.38	
	2	4	84.1	74.0	33.42	48.6	60.0 ±9.5	
		5	76.52	81.0	30.2	55.8	60.8 ±9.51	
		6	77.4	68.0	39.72	64.1	62.3 ±6.58	
Cu	**1**	1	49.8	52.1	13.8	29.88	46.1 ± 13.66	<1000
		2	61.46	48.20	72.0	24.68	61.5 ± 11.01	
		3	68.74	76.0	23.0	29.62	49.3 ± 11.02	
	2	4	76.74	59.0	59.6	33.3	64.6 ±9.89	
		5	81.0	75.0	28.6	39.76	56.1 ± 10.58	
		6	87.0	62.0	15.8	47.88	58.2 ± 13.59	
Cd	**1**	1	15.72	30.7	12.92	16.26	18.9±3.2	<100
		2	19.68	22.0	14.42	14.5	17.6±1.55	
		3	18.32	17.8	13.12	15.42	16.2±0.97	
	2	4	22.52	21.0	12.0	13.6	17.3±2.15	
		5	27.16	27.7	15.06	16.84	21.7±2.73	
		6	20.8	17.0	13.4	14.4	16.4±1.35	
Zn	**1**	1	55.6	20.6	45.6	47.8	42.4 ± 6.2	<300
		2	64.6	33.7	30.6	30.6	39.8 ± 6.77	
		3	67.7	31.7	58.8	52.8	52.7 ± 6.27	
	2	4	58.5	40.67	62.4	64.6	56.5 ± 4.45	
		5	46.6	35.8	44.6	66.7	48.4 ± 5.34	
		6	44.4	31.6	35.2	44.8	39.0 ± 2.72	
Mn	**1**	1	121.0	80.01	123.16	68.92	98.3 ±11.39	<500
		2	102.0	62	95	57.8	79.2 ±9.23	
		3	86.0	89.0	108.6	12.4	74.0 ±17.32	
	2	4	72	44	87.4	12.4	53.9 ±13.52	
		5	124	46	92	17.6	70.0±19.39	
		6	94	33	82.4	22.2	57.9 ±14.58	

In the present study, the concentration of heavy metals (Pb, Cu, Cd, and Mn) in water samples collected from Wadi EL-Rayan lakes were increased in hot seasons (summer and spring) and decreased in the cold season (winter and autumn). The increase of heavy metals concentrations in hot seasons may be due to release of heavy metals from sediment to the overlying water under the effect of high temperature and fermentation process as a result of organic matter (Elewa *et al.,* 2001) .and seasonal variation of heavy metals concentration depends on drainage water and direction of wands (Authman and Abbas, 2007).

The concentration of heavy metals in water samples collected from Wadi El-Rayan lakes followed the order Fe >Mn > Pb > Cu > Zn and Cd.

Iron (Fe) is considered as essential metal because of its biochemical and physiological role in blood cells and hemoglobin synthesis and cofactor of many enzymes (Gorur *et al*., 2012 and Edward *et al.*, 2013). However, the high amount of Fe above the physiological level of living organisms may result in iron overload (Stancheva *et al.*, 2014). The results of the present study, showed that Fe is the most abundant element in water of the two lakes, the maximum level of iron (1603.6µg/ L) observed in upper lake in winter, that's may due to agriculture drains, that's agreed with the results reported by (Shama *et al*., 2011). The level of Fe concentration in Wadi El-Rayan lakes is higher than the permissible level (300µg/l) respectively, In all sites except (site 6 in summer and sites 4, 5, 6 in spring) for iron and all sites for lead according to Egyptian Environmental law 48 decision 92/2013. The observed increase in Fe in the present study may be due to the liberation of Fe as a ferrous ion from sediment and waste from EL-Wadi drain.

Lead (pb) is a toxic event at low concentration and had no function in the biochemical process in fish. The major sources of lead in the environment are automobile exhaust, industrial wastewater, wastewater sludge and pesticides (Balba *et al.*, 1991). In the present study, the maximum value of Pb was 84.1µg/L found at the site (4) in the spring season and the minimum value was 18.5µg/L found in the cold season (autumn) at a site (1). Pb concentration in water samples collected from Wadi EL-Rayan lakes higher than the maximum permissible level (50µg/L) recommended by Egyptian Environmental low 48 decision 92/2013 in the hot season (spring) while in cold season Pb concentration was with the permissible level except at site (5) and (6).

Copper is an essential element play a vital role in enzymes activity and is necessary for the synthesis of hemoglobin (Sivaperumal *et al.*, 2007). However, when accumulated to higher amounts could pose health hazards to both animals and humans (Bashir *et al* .,2013).Copper is present in the water surface and groundwater due to the extensive use of pesticides sprays containing copper compounds for agricultural purposes. It is an essential element in human metabolism but can cause anemia, bone disorders and liver damage at excessive levels which depends upon the water hardness and pH (Taha, 2004). In the present study, the concentration of Cu in the water samples collected from Wadi El-Rayan lakes ranged from 13.8 to87.0 µg/L these values still below the maximum permissible level 1000µg/L recommended by Egyptian Environmental low 48 decision 92/2013

Cadmium is a non-essential trace metal that is potentially toxic to most fish and wildlife particularly freshwater organism (George *et al.,* 2013). Table (2) showed that Cd concentration in water samples collected from Wadi El-Rayan lakes ranged from (15.7 to 30.7µg/L) in the hot season and from (12 to 16.8µg/L) in cold season this values with the permissible limits (100µg/L) recommended by Egyptian Environmental low 48 decision 92/2013

Zinc toxicity is modified by water chemical factors including dissolved oxygen concentration, hardness, pH and temperature of the water and can also be changed through other heavy metals compounds and alkaline earth metals. High temperature tends to increase zinc toxicity, while the increase in water hardness, alkalinity and organic chelators can reduce its acute lethality and low dissolved oxygen content in water increases the toxicity of zinc (Nussey, 1998).The present results revealed that Zn concentration ranged from 20.6 to 67.7 µg/L in hot seasons and from 30.6 to 66.7 µg/L in cold season this values with the permissible level (300µg/L) recommended by Egyptian Environmental low 48 decision 92/2013

Manganese is an essential metal, and low level is necessary for human health, however, excess amount can induce oxidative stress and toxic effects in aquatic organisms (Vieira*etal*, 2011)Also, the concentration of Mn in the present study ranged from 33.0 to 123.0 µg/L in the hot season and 12.4 to 104.6µg/L in the cold season. These values with the permissible level (500µg/L) recommended by Egyptian Environmental low 48 decision 92/2013.

Our results in the present study, were in agreement with the results obtained by (Mansour and Sidky (2003) and Abd El-Satar *et al.*, 2007)

Heavy Metals Concentration in Fish Tissue:

In the present study Tilapia Spp.has been selected to assess the metals accumulation in different tissue .the concentration of Fe, pb, Cu, Cd, Zn and Mn in Tilapia Spp. caught from Wadi El-Rayan lakes was monitored seasonally from spring 2015 to winter 2016 as shown in Table (3)

Table 3: Heavy metals concentration (µg/g dry wt.) in *Tilapia* Spp. Tissues (gill-muscles-liver) collected from Wadi El-Rayan lakes.

Metal ions (µ/g dry wt.)	**Lakes**	**Organ**	**Spring**	**Summer**	**Autumn**	**Winter**	**permissible limits**
Fe	**Lake (1)**	Gill	233.5	408.7	324.8	278.7	**30µ/g dry wt (FAO,1983)**
		Muscles	73.6	131.6	128.8	165.6	
		Liver	276.3	442.5	332	382.6	
	Lake (2)	Gill	254.6	353.6	298.5	208.6	
		Muscles	163.76	181.54	116.8	108.7	
		Liver	378.5	534.9	330	349.3	
Pb	**Lake (1)**	Gill	2.12	2.73	2.81	0.61	**2.0µg/g dry wt. (Egyptian Organization (1993).**
		Muscles	1.18	0.143	1.23	0.142	
		Liver	0.61	2.12	2.332	0.54	
	Lake (2)	Gill	1.98	2.85	2.87	1.21	
		Muscles	1.21	0.78	0.48	0.164	
		Liver	2.88	1.87	1.87	0.51	
Cu	**Lake (1)**	Gill	1.65	1.64	1.65	3.65	**30µg/g dry wt. FAO (1992)**
		Muscles	0.84	0.9	1.43	1.91	
		Liver	4.8	7.77	28	11.71	
	Lake (2)	Gill	3.2	3.54	7.7	4.35	
		Muscles	1.32	1.21	1.89	2.32	
		Liver	24.0	29.01	43.2	36.3	
Cd	**Lake (1)**	Gill	1.15	0.51	2.84	1.65	**2µg/g dry wt. (FAO, 1992)**
		Muscles	0.9	N.D	1.76	2.01	
		Liver	1.42	2.52	2.15	2.6	
	Lake (2)	Gill	1.12	2.09	2.8	1.62	
		Muscles	0.87	1.37	2.03	1.21	
		Liver	2.84	2.44	3.14	2.32	
Zn	**Lake (1)**	Gill	41.7	44.7	41.7	65.71	**(40 µg/g dry wt.) (FAO/WHO, 1999)**
		Muscles	17.7	21.5	19.6	18.6	
		Liver	27	39	33.8	42	
	Lake (2)	Gill	50.7	52.9	62.7	81.6	
		Muscles	23.7	32.6	33.78	21.67	
		Liver	37.54	42.8	40.6	81.6	
Mn	**Lake (1)**	Gill	7.86	6.7	8.3	7.5	**2.5µg/g dry wt. (FAO, 1983)**
		Muscles	1.65	2.7	1.35	1.78	
		Liver	6.27	5.27	4.66	2.45	
	Lake (2)	Gill	7.5	6	8.5	8.2	
		Muscles	1.67	2.76	1.49	1.85	
		Liver	6.08	4.81	5.04	3.82	

In the present study, heavy metal concentration in the muscles of *Tilapia Spp.* Collected from Wadi EL-Rayan lakes followed the order: Fe>Zn>Cu=Mn>Cd >Pb, while in the liver follow the order: Fe >Zn >Cu >Mn>Cd>pb, and in gill follow the ranking: Fe>Zn>Mn>Cu>pb>cd Iron (Fe) is considered as essential metal because of its biochemical and physiological role in blood cells and hemoglobin synthesis and cofactor of many enzymes (Gorur *et al*., 2012). However, high amount of Fe above the physiological level in living organisms may result in iron overload . In the present study, the concentration of Fe in fish organs collected from Wadi El-Rayan lakes ranged from (73.6 to 5349.9 µg/L) Table (3) these values exceed the maximum permissible level for Fe (30µ/g dry wt.) cited by (FAO,1983). The increased accumulation of fish organs attributed to the large quantity of Fe in water, the increase of Fe in water is mainly due to the liberation of Fe as ferrous ions from sediments and waste from Wadi El- Rayan drain. Our results in the present study were in agreement with the results obtained by (Sayed and Abdel Satar, 2009) and (Mohamed and Sabae, 2015).

Lead is a non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. Lead (pb) is toxic even at low concentration and had no function in the biochemical process in fish, uptake of pb depended on exposure time aqueous concentration, pH, Temperature, salinity, diet and other parameters such as internal ion regulation capacity ,osmoregulation capacity and metabolism of species (Sorensen, 1991).In the current work, the concentration of pb in the muscle of Tilapia Spp. collected from Wadi El-Rayan lakes ranged from (0.143 to 1.23) this values below the maximum permissible limit of pb (2.0µg/g dray wt.) cited by Egyptian Organization (1993) Table (3).

Copper is an essential element play a vital role in enzymes activity and is necessary for the synthesis of hemoglobin. However, when accumulated in higher amounts it could pose health hazards to both animals and humans (Bashir *et al.*, 2013). In the present study, the concentration Cu concentration in fish tissues (liver , gill and muscles)below the maximum permissible limit (30µg/g dry wt.) recommended by FAO (1992) except in liver of Tilapia Spp. Collected from the lower lakes Table (3). These higher accumulations of essential metals such as Cu in the liver may be attributed to the binding proteins such as metallothioneins and its role in storage, metabolism and detoxification which may increase its tendency to accumulate essential metals at higher concentrations (Gorur *et al* ., 2012).

Cadmium is a non-essential element which has several toxic effects on fish .Cd can damage gill and disturbed calcium balance and may replace Zn in certain enzymes causing diseases (Gad and Youcoub, 2009). In the present study concentration of cadmium in fish muscles are ranged from 0.0 to 2.03µg/g dry wt. this value below the maximum permissible level for Cd 2µg/g dray wt. reported by (FAO, 1992).

Zinc being an essential metal is required in a certain amount for normal metabolic functions and is involved in many cellular processes either as the structural component of regulatory proteins or catalytic part of enzymes. When in excess amounts, Zn can be toxic to all living organisms including fish (Ardakani and Jafari, 2014). In the present study, the concentration of Zn in the muscles of Tilapia Spp. Collected from Wadi EL-Rayan lakes ranged from (17.7 to 33.78 µg/g dry wt.). This values within the maximum permissible level

(40µg/g) recommended by (FAO/WHO, 1999) while, in gill and liver tissues were higher than the maximum permissible limits Table (3). Our result of the present study agreement with the result obtained by (Mohamed and Sabea, 2015).

Manganese is an essential metal, and the low level is necessary for human health, however, the excess amount can induce oxidative stress and toxic effects in aquatic organisms (Vieira *et al.,* 2011). In the present study, the Manganese concentration in muscles tissue of Tilapia Spp collected from Wadi El-Rayan lakes ranged from (1.35 to 2.76 µg/g dry wt.) these values higher than the permissible limits 2.5µg/g recommended by (FAO, 1983) Also, in liver and gill tissue Manganese concentration was higher than this permissible limit. Manganese showed a wide range of variation among different tissues and different sites. The highest concentration of Mn was recorded in the gills of Tilapia fish.

The bioaccumulation of heavy metals varied between the organs (muscle, gills, and liver). Fish liver and gills (metabolic active tissues) showed significantly higher abilities for the accumulation of all metals while accumulations were lowest in fish muscle. The highest concentration of heavy metals was accumulated in liver and gill and lowest value in muscles may be due to the following reasons: Firstly the muscle does not come into direct contact with the toxicant medium as it's totally covered by skin, which helps the organism toward off the penetration of toxicant. Second, the muscles are not an active site for detoxification there for transport of heavy metals from other tissues to muscles does not be occurred (Gad and Yacoub, 2009). Moreover, metal accumulation in fish doesn't only depend on the types of metals and its accumulation in water, but also on the structure of the tissue and organs and the interaction in the environment (Karayakos and Aya, 2004). On the other hand, the highest concentration of heavy metals in the liver is related to detoxification process that takes place in this organ (Celechovska *et al.,* 2007). In general the concentration of heavy metals in Tilapia spp. Collected from Wadi El-Rayan lakes tissues found in the following order liver> Gil > muscle.

Biochemical Parameter:

Serum biochemical parameters have been described as excellent physio-pathological indicators of fish health (Roberts, 2012). These parameters have been utilized as a diagnostic tool not only for physiological alterations occasioned by stress (Wagner and Congleton, 2004) but also for detecting structural alterations in organs/systems produced by diseases.

Alteration of blood biochemistry may be indicative of unsuitable environmental conditions (temperature, pH, oxygen concentration) or the presence of stressing factors, such as toxic chemicals (Barcellos *et al.,* 2004). It is known that physiological and biochemical parameters in fish blood and tissues can change when exposed to heavy metals (Cicik and Engin, 2005). Previous studies have shown that certain metals can cause either increased or decreased levels of serum protein, cortisol, glucose, and electrolytes, as well as changes in serum enzyme activity, depending on metal type, fish species, water quality, and length of exposure (Gad, 2007), (Gad and Yacoub, 2009), (Gad 2011).

Measurement of serum biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals and has been advocated to provide early warning of potentially damaging changes in stressed organisms (Daviad *et al* ., 2010).

In the present study serum glucose and total protein concentration in Tilapia Spp. collected from Wadi El-Rayan shown in Table (4).

Table 4: Serum glucose and total protein concentration (mean ±S.E) of Tilapia Spp.fish collected from Wadi El-Rayan Lakes.

Biochemical parameter	**Lakes**	**Sites**	**Spring**	**Summer**	**autumn**	**Winter**
Glucose(mg/dl)	**Lake 1**	**Control**	67.2±2.6	63.2±3.6	74.4±1.75	71.8±0.8
		1	145.02±5.94** (115.80)	105.54±4.11** (66.99)	154.4±3.36** (107.53)	143.72±3.1** (100.17)
		2	142.2±2.27** (111.67)	123.3±4.39** (95.03)	160.6±1.93** 115.83	179.9±1.85** (150.56)
		3	200.9±3.75** (198.96)	135.3±3.24** (114.11)	163.8±1.6** 120.22	161.7±1.8** (125.15)
	Lake 2	**4**	183±2.11** (168.27)	130.3±1.84** (106.17)	160.4±2.06** 115.59	224.6±1.81** (212.81)
		5	213.4±2.06** (217.56)	146.8±2.16** (132.33)	181 ±3.22** (143.28)	261.4±5.68** (264.07)
		6	190±2.21** (182.74)	135.0±1.0** (143.0)	206.0 ± 1.92** (176.88)	251.8±4.88** (250.70)
Total protein(g/dl)	**Lake 1**	**Control**	4.24±0.07	3.91±0.06	4.4±0.07	4.06±0.08
		1	5.0±0.07 (17.92)	3.68±0.34 (-5.88)	4.69±0.07 (6.59)	4.9±0.03 (20.69)
		2	4.37±0.09 (3.07)	4.25±0.07 (8.70)	4.66±0.06 (5.91)	5.06±0.3 (24.63)
		3	4.2±0.07 (-0.94)	4.2±0.14 (7.42)	4.59±0.03 4.32	3.76±0.16 (-7.39)
	Lake 2	**4**	4.54±0.16 (7.08)	4.98±0.06 (27.37)	5.0±0.11 (13.64)	4.71±0.12 (16.01)
		5	5.16±0.15 (21.70)	4.3±0.08 (9.97)	6.0±0.16 (36.36)	5.69±0.07 (40.15)
		6	4.2±0.19 (-0.94)	5.0±0.07 (27.88)	5.28±0.09 (20.00)	4.41±0.07 (8.62)

- ** Highly significant difference ($p \leq 0.01$) as compared with control - Data between brackets represent the %of change from control

-Data are represented as (M±S.E) of 7 fish

Blood glucose is a sensitive and reliable indicator of pollutants causing environmental stress in fish and a sensitive indicator of environmental stress for any chemical pollutants (Banaee, 2012). In the present study, the serum glucose level of Tilapia Spp.fish collected from different sites and seasons in Wadi EL-Rayan lakes showed a highly significant increase ($P \leq 0.01$) as compared to control groups.The observed increase serum glucose concentration (hyperglycemia) indicated that fish generated more glucose to produce the energy to combat the stress induced by environmental pollution. Also, Elevation in serum glucose level that was observed might be have resulted from an increase in glucogenesis and glycogenolysis as well as inhibition of glucogenolysis and glycogenesis during stress (Yekeen and Fawole, 2011).

In teleosts fish as in mammals, the stressful situation is characterized by an increase of secretion of both catecholamines and corticosteroids hormones which induced marked changes in the carbohydrate energy reserves of fish, both hormones are known to produce hyperglycemia in animals. The hyperglycemic condition in this study may be related to increased secretion of these hormones which causes the breakdown of liver glycogen. The present findings are in agreement with previous reports of the increased level of plasma glucose on exposure to heavy metals (Cicik and Engin, 2005, Gad and Yacoub, 2009 and Abedi, *et al*., 2013). The observed increase in serum glucose level of Tilapia fishes collected from Wadi El-Rayan lakes was in agreement with the results observed in O.niloticus collected from El-Khadrawia drain in Quesna EL-Manofya governorate. (El-Gaar, 2015), and in *O. niloticus* collected from Rosetta branch of River Nile (Moustafa, 2017) which polluted by heavy metals.

In the present study, serum Total protein in Tilapia spp. Collected from Wadi El-Rayan lakes showed a narrow variation compared to the control group this variation statistically not significant.

In the present study serum creatinine and urea concentration in Tilapia Spp. collected from Wadi El-Rayan shown in Table (5).

Table 5: Serum creatinine and urea concentration (mean ±S.E) of Tilapia Spp.fish collected from Wadi El-Rayan Lakes.

Biochemical parameter	**Lakes**	**Sites**	**Spring**	**Summer**	**autumn**	**Winter**
Creatinine (mg/dl)		Control	0.4±0.01	0.54±0.02	0.34±0.01	0.37±0.02
	Lake 1	1	0.85±0.02** (112.50)	1.08±0.09** (100.00)	0.9±0.03* (164.71)	0.48±0.02 (29.73)
		2	0.61±0.03* (52.50)	0.83±0.02* 53.70	1.23±0.05* (261.76)	0.54±0.03* (45.95)
		3	0.81±0.02** (102.50)	0.67±0.01 (24.07)	0.76±0.01** (123.53)	0.87±0.02** (135.14)
	Lake 2	4	0.6±0.01* (50.00)	0.60±0.02 (11.11)	1.96±0.08* *(476.47)	0.6±0.01** (62.16)
		5	0.59±0.02* 47.50	0.66±0.3 (22.22)	0.78±0.01** (129.41)	0.44±0.03 (18.92)
		6	0.68±0.02** (70.00)	0.84±0.02* 55.56	0.61±0.02** (79.41)	0.55±0.03* (48.65)
Urea (mg/dl)		Control	15.3±1.04	8.55±0.37	10.9±1.78	11.8±1.18
	Lake 1	1	24.4±3.46* (59.48)	48.38±2.06** (465.85)	30.96±1.19* (183.26)	26.1±1.17** (106.43)
		2	24.32±3.86* (58.95)	40.04±3.57** (368.30)	20.06±0.9** (83.53)	27.22±2.0** 105.75)
		3	31.68±2.8** (107.6)	41.14±2.14** (369.47)	21.94±1.45** (100.73)	32.16±1.05** (168.02)
	Lake2	4	27.48±3.49** (79.61)	29.54±1.1** (245.50)	19.56±0.66** (78.96)	22.38±0.86** (132.49)
		5	32.45±1.36** (112.09)	39.12±1.29** (357.54)	20.95±1.58** (91.67)	34.0±2.44** (174.53)
		6	28±3.74** (83.01)	32.98±1.17** (285.73)	22.97±1.24 (110.66)	36.17±2.04** (136.89)

** Highly significant difference ($p \leq 0.01$) as compared with control * Significant difference ($p \leq 0.5$) as compared with control -Data are represented as (M ±S.E) of 7 fish

-Data between brackets represent the %of change from control

Plasma urea and creatinine were useful in the diagnosis of renal function impairment, renal insufficiency, renal tubular necrosis, muscle tissue damage as well as impaired nitrogen metabolism (Murray *et al*., 1990).

In the present study, serum creatinine and urea level showed highly significant increased ($P \leq 0.01$) for urea and significant increase ($P \leq 0.05$) for creatinine in Tilapia spp. collected from different sites of Wadi EL-Rayan lakes. The observed increase in serum creatinine and urea in the present study may be due to kidney damage which results in reduced renal blood flow with a reduction in glomerular filtration rate, resulting in azotemia characterized by an increase in blood urea nitrogen and creatinine (Gad, 2007). This results support by results were obtained by Abdel-Tawwab *et al.*, (2012) and (2013) who found creatinine increases in Nile tilapia and common carp, respectively, due to Zn toxicity. Urea in fish is synthesis by the liver and excreted primarily by the gills rather more the kidney. So in the present study, the elevation of urea level may be attributed to gill dysfunction (Stoskoph, 1993). It is shown that increased blood urea could occur at times of impaired kidney function, liver diseases and cardiac arrest (Abdelmoneim *et al*., 2008). Cawson *et al.,* (1982) noted that plasma levels of urea and creatinine depend largely on glomerular function. However, the rise in urea, uric acid and creatinine could be due to increased muscular tissue catabolism, decreased urinary clearance by the kidney, increased synthesis or decreased degradation of these compounds Also, the observed increase in serum urea and creatinine could be attributed to the action of accumulated recorded pollutants on the glomerular filtration rate, which caused pathological changes in the kidney. These results may be also supported by the findings of (Authman and El-Sehamy, 2007) who illustrated that exposure of fish to high concentrations of heavy metals and pesticides led to the disintegration of the renal epithelial cells, displacement of nuclei, shrinkage of glomeruli, breakdown of Bowman's capsules and heavy infiltration by inflammatory cells.

In the present study serum Total cholesterol and triglycerides concentration in Tilapia Spp. collected from Wadi El-Rayan shown in Table (6).

Table 6: Serum Total cholesterol and triglycerides concentration (mean ±S.E) of Tilapia Spp. fish collected from Wadi El-Rayan Lakes

Biochemical parameter	**Lakes**	**Sites**	**Spring**	**Summer**	**autumn**	**Winter**
Total Cholesterol (mg/dl)	**Lakes**	**Control**	178.3±2.91	180.64±2.41	177.38±2.23	180.25±1.15
	Lake 1	**1**	189.65±3.1 (6.37)	176.82±3.46 (-2.02)	218.06±2.16 (22.93)	227.66±2.26 (26.30)
		2	218±5.05 (22.24)	230.7±3.51 (27.82)	220.5±4.14 (24.29)	424.7±21.1** (135.60)
		3	219.7±4.05 (23.24)	202.1±6.57 (12.00)	199.2±7.75 (12.28)	338.7±10.01** (87.88)
	Lake 2	**4**	192.9±7.59 (8.20)	221.6±0.33 (22.79)	245±2.58 (38.12)	361.9±22.7** (100.77)
		5	175.60±4.31 (-1.54)	172.4±5.55 (-4.44)	323.5±3.76** (82.39)	310.3±20.26** (72.13)
		6	142.8±3.58 (-19.93)	181.8±1.83 (0.72)	257.1±7.88* (44.9)	311.8±1.15** (72.97)
Triglycerides (mg/dl)		**Control**	160.40±1.8	158.84±2.59	155.24±2.96	165.38±1.75
	Lake 1	**1**	221.88±3.46 (38.33)	184.95±2.0 (16.44)	168.87±3.09 (8.78)	308.75±3.35** (86.69)
		2	252.7±3.86* (57.53)	228.3±3.57* (43.70)	202.5±4.49 (30.44)	418.4±4.81** (152.99)
		3	263.7±2.8** (64.37)	222.4±3.63 (39.98)	177.48±1.59 (14.33)	431±1.02** (160.62)
	Lake 2	**4**	321.5±3.49** (100.45)	172.3±4.11 (8.47)	174.7±1.54 (12.51)	465.0±8.58** (181.15)
		5	279.7±3.6** (74.35)	245.4±3.39* (54.47)	216.6±3.89 (39.53)	505.8±5.92** (205.84)
		6	277.4±3.74** (72.93)	221.6±6.4 (39.51)	223.2±3.08* (43.75)	481.6±4.73** (191.20)

** Highly significant difference (p≤0.01) as compared with control * Significant difference (p≤0.5) as compared with control -Data are represented as (M ±S.E) of 7 fish

-Data between brackets represent the %of change from control

The main lipid classes of plasma are serum Total cholesterol and triglycerides. The level of triglycerides and total cholesterol in serum of fish have been reported to be moderately sensitive to environmental pollutant but the direction of change in these parameters seems to be dependent on many factors, such as the types of contaminant, the concentration, mode of its action, duration of exposure and fish species (Heath, 1995).

In the present study serum Total cholesterol and Triglycerides of Tilapia spp. Collected from Wadi El-Rayan lakes were increased significantly ($P \leq 0.05$) as compared to control fish. The increased value of plasma Total cholesterol and triglycerides indicated retardation of fat metabolism. The increase of total cholesterol observed in the present study may be due the increase of lipids peroxides formation induced by the effect of pollutants (heavy metals and pesticides) as previously reported by (Mousa, 2004), also the destruction of the liver cells and other organs due to the effect of environmental pollution increase the levels of total cholesterol and triglycerides in the plasma (Shalaby *et al.,* 2007). The rise in plasma triglycerides is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up triglycerides (Metwally, 2009).Similar increase in plasma Total Cholesterol and Triglycerides were noted by (Shalaby *et al*., 2007) in *O. niloticus* exposed to butataf herbicide, by (Metwally, 2009) in *O. niloticus* exposed to copper and zinc and by (Mohamed and Gad, 2009) in *O. niloticus* exposed to Zn and / or Cadmium. The present results are in agreement with the findings of O¨ ner *et al.,* (2008) who found that cholesterol concentrations in the serum of metal-exposed *O. niloticus* generally increased when compared to that of the control value. They concluded that the concentrations of cholesterol, an essential structural component of membranes and the precursor of all steroid hormones, may increase due to liver and kidney failure causing the release of cholesterol into the blood.

In the present study serum AST and ALT activities in Tilapia Spp. collected from Wadi El-Rayan shown in Table (7).

Transaminase AST and ALT are important enzymes known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological conditions(Manjunatha *et al* ., (2015). These transaminases belong to the serum non-functional or tissue-specific enzymes which are normally localized within the liver and other organs. Therefore, their presence in the serum of the collected fish may give information and evidence for injury of the tissue or organ dysfunction (Fernandes *et al*., 2008). The increase of AST and ALT values in the fish reveal enzymes exporting from the liver into the bloodstream (Perez-Rostro *et al.,* 2004). According to (Chen *et al.,* 2004) increased levels of these two enzymes in tilapia are associated with hepatic injury. The activity of AST and ALT enzymes in the blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (Svoboda, 2001).

In the present study, serum ALT enzyme activity of Tilapia spp. collected from Wadi El-Rayan lakes was a highly significant increase in Table (4) ($P \leq 0.01$) in all sites for all season except site (1) in autumn showed a significant increase ($P \leq 0.05$) as compared to the control group. Also, serum AST was increased significantly ($P \leq 0.05$) in most sites in all season and showed a highly significant increase (P≤0.01) in sites (4 in spring) as compared to the control group. Elevations in the activities of plasma (AST) and (ALT), in Tilapia spp. from Wadi El-Rayan Lakes reflect hepatic and myocardial impairment, leading to extensive liberation of the enzymes into the blood circulation. Al-Attar, (2005) reported that the elevation of serum (AST) and (ALT) may be due to liver dysfunction. In addition, the increase of serum (AST) and (ALT) may be attributed to the hepatocellular damage or cellular degradation, perhaps in liver, heart or muscle (Mohamed and Gad, 2009). The observed increase in the activities of ALT &AST was in agreement with the results obtained by (Firat and Kargin 2010) who found increases in ALT and AST activity in Nile tilapia serum caused by the individual and combined effects of exposure to Zn and Cd. (Abdel-Tawwab *et al*, 2012 and 2013) found significant increases in ALT and AST activity in Nile tilapia and common carp, respectively, when exposed to different Zn concentrations. Also, the observed increased in serum AST and ALT in the present study in according with that reported in Tilapia Zilli, Solea Vulgaris and Mugli Capita collected from lake Qaroun (Mohamed and Gad 2009), in Tilapia spp.collected from Wadi EL-Rayan lake (Sabea and Mohamed 2015),and serum of *O. niloticus* collected from Rosseta branch of River Nile (Moustafa, 2017).

Table 7: Serum serum AST and ALT activities (mean ±S.E) of Tilapia Spp. fish collected from Wadi El-Rayan Lakes

Biochemical parameter	**Lakes**	**Sites**	**Spring**	**Summer**	**autumn**	**Winter**
AST (u/l)		**Control**	155±2.04	144±3.19	155±3.96	149±3.01
	Lake 1	**1**	176.92±1.96 (13.92)	187.57±2.5 (29.99)	145.18±1.5 (-6.40)	216.63±2.93* (45.58)
		2	207.9±4.12 (33.84)	191.2±3.07 (32.52)	161±5.46 (3.80)	193.9±1.91 (30.31)
		3	232±4.39* (49.39)	143.8±0.92 (-0.32)	131.2±3.58 (-15.44)	205.6±4.07 (38. 2)
	Lake 2	**4**	254.9±4.19** (64.10)	191.5±3.36 (32.70)	152.4±3.07 (-1.77)	156.2±3.4 (4.94)
		5	193.8±2.03 (24.79)	229±2.85* (58.71)	192.8±3.07 (24.28)	224.4±4.12* (50.81)
		6	242±5.18* (55.85)	119.7±3.43 (-17.03)	240.2±3.06* (54.85)	177.2±2.7 (19.09)
ALT (u/l)		**Control**	71±3.01	57.1±1.89	66.8±1.82	57.8±2.28
	Lake 1	**1**	122.88±2.36** (72.97)	118.84±1.27** (108.13)	94.86±1.63* (41.92)	112.13±1.53* (93.86)
		2	150. 5±1.51** (111.89)	147.1±1.89** (157.62)	142.6±1.94** (113.35)	101.6±2.21** (75.57)
		3	141.6±1.88** (99.38)	141.0±1.5** (146.97)	171.8±1.62** (157.09)	112.3±2.45** (94.16)
	Lake 2	**4**	141.6±2.42** (99.30)	143.5±1.02** (151.38)	204.1±1.32** (205.34)	117.8±4.68** (103.67)
		5	119.7±4.71** (68.47)	154.2±0.95** (169.98)	171.5±4.38** (156.61)	128.1±3.02** (121.44)
		6	130.4±3.29** (83.53)	129±3.53** (125.95)	177.2±2.18** (165.05)	119.6±2.91** (106.78)

** Highly significant difference (p≤0.01) as compared with control

* Significant difference (p≤0.5) as compared with control

-Data are represented as (M ±S.E) of 7 fish

-Data between brackets represent the %of change from control

CONCLUSION

This study was carried to evaluate the seasonal variation of investigated heavy metals in the water samples, in addition, to assess some biochemical parameters and heavy metals in tissues of Tilapia Spp. fish sample collected from Wadi El-Rayan lakes. The results revealed that the two lakes suffer from the different degree of metal concentration and the lower lake more polluted than the upper lake. the concentration of heavy metals (Pb, Cu, Cd and Zn) in the muscles (edible part) within the maximum permissible levels while the concentration of Fe and Mn higher than the maximum permissible level where consumption of Tilapia Spp. Fish inhabit in Wad El- Rayan lakes can cause metal accumulation in the human body.

RECOMMENDATIONS

Treating the different waste (domestic and agricultural) before being discharged from El-Wadi drain to the lakes.

Continuous monitoring of water quality seasonally and regional of the water lakes.

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