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Assessment of environmental and pollution level in water and insect's fauna of freshwater habitat

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Abstract

The environmental problems have raised the interest for bio-indicators able to reflect their environment. Among these life forms, the insects may contribute to a viable evaluation of the sustainability degrees. The Egyptian Mediterranean coast exhibits six lakes which are situated along the Nile Delta coast; Northern Delta lakes; Manzala, Borollus, Edku, and Mariut, and to the east of the Suez Canal, Port-Fouad and Bardawil. The current study aimed to evaluate different heavy metals in aquatic insects *Ochthebius (Asiobates) rugulosus* Wollaston 1857, taken from Lake Manzala, and to estimate their effect on the oxidative stress markers to employ insects as sensitive bio-monitors for environmental pollution. A lot of fluctuations were observed affected by the poor connection of the water body of the lake Manzala. The mean level concentrations of Pb, Cd, Cu and Zn in loc.#1, whereas; 0.98, 3.02, 0.35 and 1.99 $\mu\text{g/l}$; respectively with the arrangement of $\text{Cu} < \text{Pb} < \text{Zn} < \text{Cd}$ in location #1 whereas it was reported as 1.8, 3.64, 1.32 and 2.2 $\mu\text{g/l}$ in location#2, giving the arrangement of $\text{Pb} < \text{Cu} < \text{Zn} < \text{Cd}$ in loc.#2. The mean concentration level of Pb in both locations was less than that recommended by the USEPA, 2005 (2.5-65 $\mu\text{g/l}$), Cd in the present study was higher than that of the water quality criteria; (0.2-2 $\mu\text{g/l}$) recommended by USEPA, 2005 in both locations in Lake Manzala. The pH values of water were alkaline throughout the lake. It is concluded from the obtained data that the southeastern region showed worse quality parameters. The pH of the water lake (loc.1; 7.7), (loc.#2; 7.3) was found to be slightly alkaline throughout the spring (March-May, 2018). The pH was within the prescribed limits (6.5-9) of USEPA, 2005. In the current study, Cu and Zn concentration in *Ochthebius (Asiobates) rugulosus* Wollaston 1857, in Lake Manzala ranged from (0.2-0.1 $\mu\text{g/g}$, 0.07-0.09 $\mu\text{g/g}$). The mean concentration of heavy metal was significantly ($p < 0.05$) different in water samples collected from Lake Manzala, Spring; 2018. The arrangement of elements in the insect tissue was as; $\text{Cu} > \text{Pb} > \text{Zn} > \text{Cd}$. There was a significant increase in the mean concentrations level of ALT activity in insects from loc. #2; 42.6 (U/mg protein), while it was 37 (U/mg protein) in that of loc.#1. The mean concentration level of

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AST activity in insects from loc.#1 was 50.8 (U/mg protein), while it was 59 (U/mg protein) in that of loc.#2. A significant decrease in GPx activity in insects from loc.#2 was found as, 68.74 (mU/mg protein), while it was 77.4 mU/mg protein in that of loc.#1. The results of the present study showed that there was a significant decrease in GPx activity in samples collected from loc.#2 compared with that of loc.#1 telling that insects in loc.#2 are under stress of sewage pollution (Bahr El Baqur) drain. The Pb in insect tissues in loc.#1 is positively correlated with Zn in insect tissues, TP, MDA, and GPx as $r=0.693$, $r=0.854$, $r=0.873$, and $r=0.644$; respectively. Cd in insect tissues is positively correlated with AST, TP, MDA and GPx as $r=0.870$, $r=0.690$, $r=0.666$ and $r=0.737$; respectively. Cu in insect tissues is negatively correlated with AST as $r=-0.739$. Zn in insect tissues is positively correlated with TP, MDA, and GPx as $r=0.742$, $r=0.810$, $r=0.951^*$; respectively. TP is highly positively correlated with MDA and GPx as $r=0.983^*$, and $r=0.840^*$; respectively whereas, MDA is highly positively correlated with GPx as $r=0.882^*$. In loc.#2, Pb in insect tissues is negatively correlated with Cu in insect tissues, TP, MDA and GPx as $r=-0.716$, $r=-0.916^*$, $r=-0.878^*$ and $r=-0.704$; respectively. Cd in insect tissues is negatively correlated with MDA as $r=-0.655$. Cu in insect tissues is positively correlated with TP and GPx as $r=0.806$ and $r=0.658$; respectively. Zn in insect tissues is negatively correlated with AST as $r=-0.813$.

Key words: Ecology- environment- fauna- pollution- lakes- beetles - Manzala.

Introduction

Manzala is the largest coastal lake in Egypt which is shallow brackish extending between the Damietta Nile River branch and the Suez Canal with a maximum length of 50km along the Mediterranean sea (Ahmed *et al.*, 2009). Wastewater effluents are flowing into Manzala Lake from nine major drains and canals. Fraskur, AlSarw, Baghous, Abu Garida, Bahr El Baqur, are the most important of them. Manzala Lake is suffering environmental changes where a human activity such as the construction of fish farms, roads, and agricultural purposes have changed the lake to a semi-closed subbasin (Elmorsi *et al.*, 2017). The pH of water samples was within the water criteria limits (6.5-9) (GAFRD, 2015). The pH values were generally high in northern sites influenced by the inflows from the seawater inlets (Abdo, 2004).

Aquatic Coleoptera constitutes a significant part of freshwater habitats. Approximately 25 families in three of four suborders of Coleoptera are typically aquatic in some of their life stages (Balke, 2005). Among these are the minute moss beetles of the family Hydraenidae. These are small beetles, 1–3 mm long (Jäch, 1995). Aquatic insects might be adversely influenced by heavy metals in the environment. The toxicity is grandly a function of the water chemistry and sediment composition in the surface water system (Mahler and Van Metre, 2006). The insects are responsible for numerous processes in the ecosystem and their loss can have negative impacts on entire communities (Gray *et al.*, 1999; Nicholasa *et al.*, 2007). The toxicity of metals is the capacity of a metal to bring about undesirable impacts on aquatic organisms

(Rasmussen and Sorensen, 2000). The poisonous quality increments when the medium ends up acidic (Mukhopadhyay and Hashim, 2011). Heavy metals are brought into aquatic systems, rivers, lakes through dumping wastes and domestic effluents (Al-Yousuf and El Shahawi, 2000; Radwan *et al.*, 2012; Radwan *et al.*, 2014; Radwan, 2016; Radwan *et al.*, 2016; Radwan *et al.*, 2017; Radwan *et al.*, 2018).

Substantial metals defile the earth due to their non-degradable nature (Ayanabenro and Babalola, 2017), the wastewater goes into the soil (Easa and Abou Rayan, 2010). Metalloids have caused harm to the biological community (Gaur *et al.*, 2014). Agrarian spillovers contain contaminations from herbicides and pesticides, which effectively affect the waterway and the general population utilizing it (Dakkak, 2016). Water contamination is the tainting of regular water bodies by physical, compound pathogenic, or microbial substances (Chaturvedi *et al.*, 2013; Bahnasawy and Khidr, 2011). It has been proposed that water contamination is the basic overall reason for some sicknesses (Sa'eed and Mahmoud, 2014; Safari *et al.*, 2014).

The impacts of heavy metals on different insects revealed, growth inhibition, reduced reproduction, developmental abnormalities, and decreased hatchability (Sidanchandra and Crane, 2000; Bijita *et al.*, 2014). The Egyptian Mediterranean coast exhibits six lakes or lagoons which are situated along the Nile Delta coast; Northern Delta lakes Manzala, Borollus, Edku, and Mariut, and to the east of the Suez Canal, Port-Fouad and Bardawil (Mehanna, 2009).

The contamination sources occur due to anthropogenic development. Since lakes are basic sinks for a few toxic substances gotten from their watershed, the lake can give rich information on the lake's overwhelming metal tainting and additionally on natural changes in the including catchments (Stephane *et al.*, 2004; Thevenon *et al.*, 2011). Metals apply their poisonous quality in living animals by different diverse systems, especially their intrusion in oxidative biochemical reactions through the advancement of receptive oxygen species (Goyer and Clarkson, 2001; Viarengo, 2003).

The ecological issues have raised the enthusiasm for bio-pointers ready to mirror their condition. Among these living things, the creepy crawlies may add to a reasonable assessment of the maintainability degree (Tylianakis *et al.*, 2004; Lopes and Vasconcelos, 2008). The resilience of oceanic living beings to overwhelming metals has been illuminated by the metallo-thionein arrangement in various amphibian creatures (Bisthoven and Janssens, 1998). The water scrounger creepy crawlies are known among entomologists particularly they are frequently bounteous in many sorts of waters, possess waterways and distinctive environments (Hansen, 1999; Short and Heabuer, 2006). Various bugs are used for bio-sign because as they are polyphagous predators (Crowson, 1981).

The sources of ROS generation incorporate progress metals (Klaunig and Kamendulis, 2004). Superoxide anion is a type of responsive animal varieties (Miller *et al.*, 1990). Heavy metal particles, for example, copper, and lead, can incite the age of responsive radicals. The cancer prevention agent parameters, for example, glutathione peroxidase and lipid peroxidation are utilized as biomarkers in an assortment of living beings (Cheung and Zheng, 2001). The most as often as possible archived and soonest result of the substantial metal poisonous quality in bug's cells is the overproduction of ROS (Shahid *et al.*, 2014). MDA is considered the most mutagenic result of lipid peroxidation. MDA has been broadly utilized for a long time as an appropriate

biomarker for lipid peroxidation, it is a standout amongst the most well-known and solid markers that decide oxidative worry in clinical circumstances (Giera and Ioan-Facsinay, 2012). At the point when oxidative pressure happens, cells attempt to adjust the oxidant impacts by an enactment of qualities encoding cautious catalysts factors, and basic proteins (Dalton and Sbertzer, 1999). Oxidation of methionine deposits of proteins causes conformational changes, protein unfurling, and corruption (Lyra and Cairns, 1997).

The presence of contaminants in the aquatic environment poses significant toxicological risks to many organisms and finds its way to the food chain, threatening the ecological balance and the biodiversity of nature (Dar *et al.*, 2014). Most of the chemicals used on land enter the aquatic environment via wastewater from agricultural and domestic sources including municipal sewage treatment plants (Ohe *et al.*, 2004). Enzymatic antioxidant defenses involve superoxide dismutase, glutathione peroxidase, and catalase (Valko and Leibfritz, 2007). The glutathione peroxidase system contains many components, including the enzymes glutathione peroxidase (Schafer and Buettner, 2001). The ROS are removed in cells by antioxidant systems (Cadenas, 2004; Li and Kong, 2009). Several antioxidant responses, including oxidative stress biomarkers and antioxidant enzyme activities, are utilized in environmental risk assessment (Song *et al.*, 2006). Amino transferases are enzymes that intermediate the transfer of an amino group from the amino donor to the acceptor for chiral amino acid or amine synthesis (Hollmann and Arends, 2011). The adjustments in protein are essential to demonstrate the defenselessness of organ systems to contaminations by changing their capacity (Fahmy, 2012). Tissue protein content has been recommended as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998). The current study aimed to evaluate different heavy metals in aquatic insects taken from Lake Manzala. Estimate their effect on oxidative stress markers to estimate the probability of employing insects as sensitive monitors for environmental contaminates.

Material and methods

The aquatic insect in the present study is the aquatic beetle; *Ochthebius (Asiobates) rugulosus*.

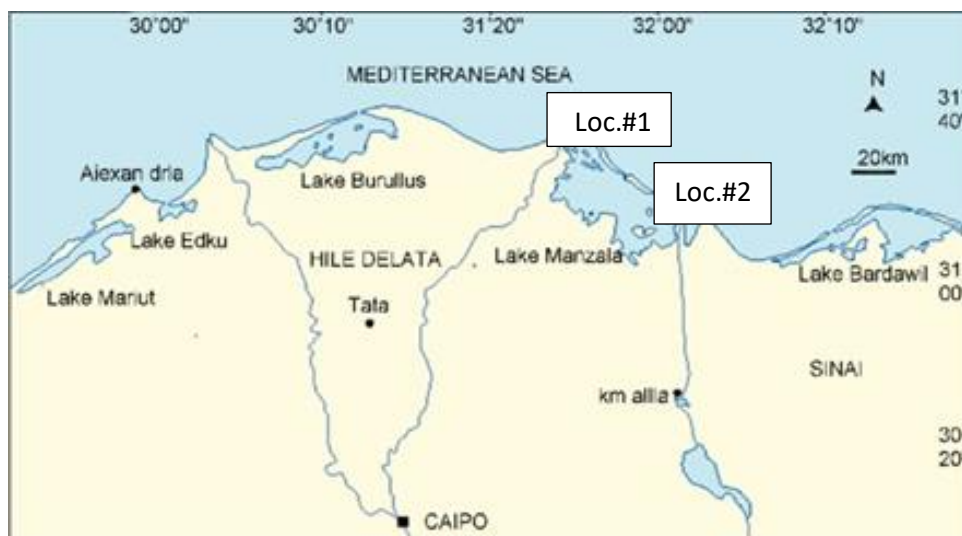


Fig. (1): Map of Manzala Lake (loc.#1; west of the lake and loc.#2; east of the lake)

Beetles and water were sampled from Lake Manzala in Spring (March-May, 2018); (the collection is bi-week) location #1 and #2 (Fig. 1). This area is occasionally affected by the seawater entering the lake. Samples of the water beetle, each consisted, of 20/time/location were collected from the studied Lake Manzala from each location/5 times replicates. Insects were then transferred to the laboratory within two hours where sorting. Sorted insects were washed in running water to remove any debris. Sorted insects were then kept at -20 °C until processing. Water samples were filtered in the field using a polypropylene syringe fitted with a 0.45µm Millipore acetate filter. Concentrations of heavy metals (lead, cadmium, copper, zinc) were measured in filtered lakes water according to (Riley and Taylor, 1968) using graphite furnace atomic absorption spectroscopy (Berkin- Elmer model 2380). The concentration level (µg/g insect tissues) of; lead and cadmium, copper, zinc were determined in dry insect samples (five replicates collected from the two locations, lake Manzala according to Loring and Rantala (1992).

The pH and alkalinity of the lake water were measured by standard methods for the analysis of natural and treated wastewater described by the American Public Health Association (1975). The water of each location was analyzed to determine the chemical composition at the beginning of the study and was conducted with insect sampling. Five random

water samples were collected from each sampling location about 20 cm below the water surface to avoid floating matter. Determination of Aspartate aminotransferase and Alanine aminotransferase activity was done according to (Tietz, 1976). Determination of Malondialdehyde (MDA) concentration was according to (Ohkawa *et al.*, 1979). Determination of glutathione peroxidase activity was according to (Paglia and Valentine, 1967) and determination of the total protein content was according to Lowry *et al.* (1951). Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov, Shapiro, and D' agstino tests were used to verify the normality of distribution of variables, Student t-test was used to compare two groups for normally distributed quantitative variables. Pearson coefficient was used to correlate between quantitative variables. Significance of the obtained results was judged at the 5% level (Bauley 1981; Lesilie *et al.*, 1991; Puri, 2002)

Results:

The obtained data from table (1), fig. (2) showed that the mean level concentrations of Pb, Cd, Cu, and Zn in loc.#1, whereas; 0.98, 3.02, 0.35, and 1.99 µg/l; respectively with the arrangement of Cu < Pb < Zn < Cd in location #1 whereas it was reported as 1.8, 3.64,

1.32 and 2.2 $\mu\text{g/l}$ in location#2, giving the arrangement of $\text{Pb} < \text{Cu} < \text{Zn} < \text{Cd}$ in loc.#2. The mean concentration level of Pb in both locations was less than that recommended by the USEPA, 2005 (2.5-65 $\mu\text{g/l}$), Cd in the present study was higher than that of the water quality criteria; (0.2-2 $\mu\text{g/l}$) recommended by USEPA, 2005 in both locations in Lake Manzala.

The parameters of water samples collected from the two locations Lake Manzala compared with that of the United States Environmental Protection Agency USEPA (2005). The mean concentration level of Cu and Zn in the present study showed a low level of

concentration than that recommended by USEPA, 2005 (9-13 $\mu\text{g/l}$) and (120 $\mu\text{g/l}$); respectively in both locations of Lake Manzala during spring (March-May 2018). The data cited in a table (1) indicated that the mean concentration level of Pb, Cu, Zn in lake Manzala were less than the standard criteria recommended by USEPA, 2005, only Cd was reported in a higher concentration level than the criteria recommended by USEPA, 2005. The data represented in table (1) showed that the mean concentration of heavy metal was significantly ($p < 0.05$) different in water samples collected from Lake Manzala, Spring, 2018.

Table (1a, b): Trace metal concentrations ($\mu\text{g/L}$) in water samples from Lake Manzala in Spring (March-May, 2018):

Table1.a	Loc.#1 (West side of the lake)	t-test	p	USEPA, 2005 (freshwater standard criteria)
Pb $\mu\text{g/l}$ (Mean)	0.98 \pm 0.5*	2.687*	0.028*	2.5-65
Cd $\mu\text{g/l}$ (Mean)	3.02 \pm 1.9	0.479	0.645*	0.25-2
Cu $\mu\text{g/l}$ (Mean)	0.35 \pm 0.3*	3.017*	0.017*	9-13
Zn $\mu\text{g/l}$ (Mean)	1.99 \pm 1.7	0.190	0.854	120

Data were expressed by using mean \pm S.D. Statistically significant: at $p^* \leq 0.05$

Table1.b	Loc.#2 (East side of the lake)	t-test	P	USEPA, 2005 (freshwater standard criteria)
Pb $\mu\text{g/l}$ (Mean)	1.8 \pm 0.5*	2.687*	0.028*	2.5-65
Cd $\mu\text{g/l}$ (Mean)	3.64 \pm 2.2*	0.479	0.645	0.25-2
Cu $\mu\text{g/l}$ (Mean)	1.32 \pm 0.7*	3.017*	0.017*	9-13
Zn $\mu\text{g/l}$ (Mean)	2.2 \pm 1.7*	0.190	0.854	120

Data were expressed by using mean \pm S.D. Statistically significant: at $p^* \leq 0.05$

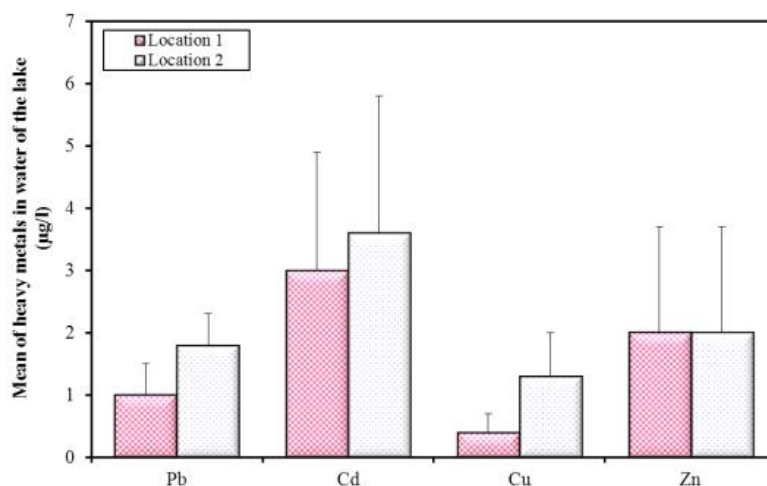


Fig.2: The mean concentration level of some heavy metals in Lake Manzala water in Spring, 2018.

Table (2), fig. (3) represents the pH value, the alkalinity, the obtained data showed that the mean value of pH in loc.#1 was 7.7(mg/L), whereas in loc.#2 it was 7.3(mg/L).USEPA (2005), reported the pH concentration level in freshwater ranges between (6.5-9), giving the water lake is within that range and that water of lake Manzala is on the alkaline side.

There was a significant increase in alkalinity in loc.#2 as 17.98 (mg/L), while that of loc.#1 was 14.9 (mg/L) table (2) and that is less than the standards for USEPA (2005). The USEPA, 2005 criteria are (2000), the water of the lake is less than that recommended criteria.

Table (2): Physico-chemical characteristics of water collected from lake Manzala (loc.#1 and loc.#2):

	Loc.#1	Loc.#2	t-test	P	USEPA, 2005 freshwater criteria
pH	7.7±0.1*	7.3±0.2*	4.583*	0.002*	6.5-9
Alk	14.9±1.1*	17.98±0.3*	6.256*	0.002*	2000

Data were expressed by using mean ± S.E. Statistically significant at $p^* \leq 0.05$

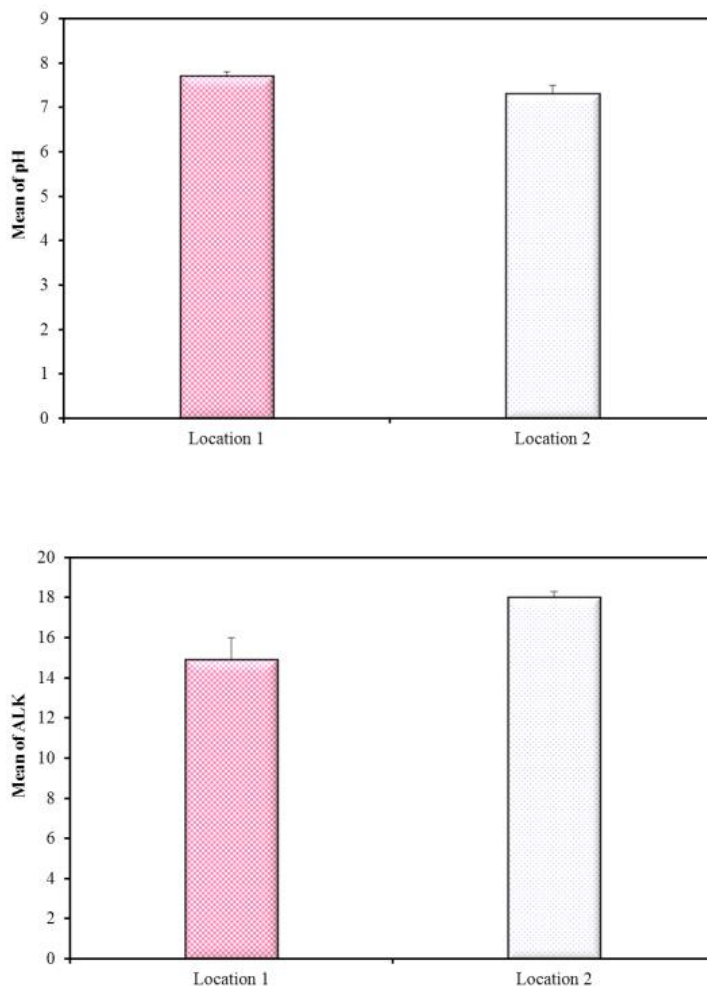


Fig. (3 a, b): Represents the pH and Alkalinity of the Lake Manzala water in Spring, 2018

Table (3): Heavy metals mean concentration levels in insects ($\mu\text{g/g}$ dry weight) collected from Lake Manzala (Spring, 2018)

	Loc.#1	loc.#2	t-test	p
Pb $\mu\text{g/g}$	0.06 \pm 0.01	0.12 \pm 0.02	1.713	0.125
Cd $\mu\text{g/g}$	0.03 \pm 0.01	0.03 \pm 0.01	0.000	1.000
Cu $\mu\text{g/g}$	0.20 \pm 0.30	0.10 \pm 0.01	0.664	0.543
Zn $\mu\text{g/g}$	0.07 \pm 0.02	0.09 \pm 0.03	1.241	0.250

Data were expressed by using mean \pm S.E. Statistically significant at $p^* \leq 0.05$

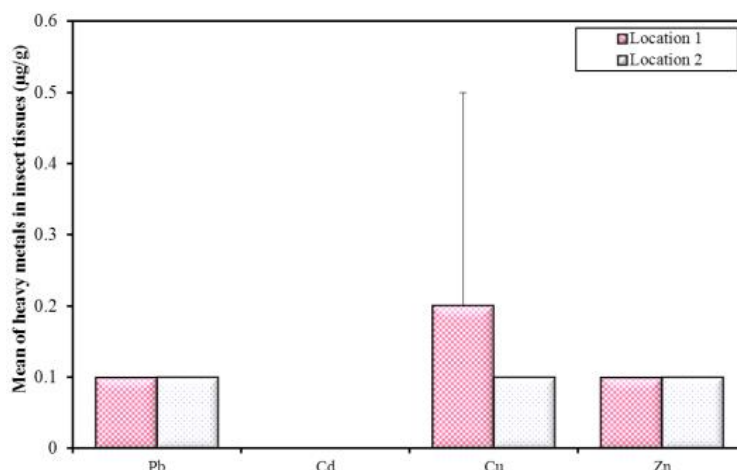


Fig. (4): Histogram represents the heavy metals mean concentration levels in insects collected from Lake Manzala in Spring, 2018.

The obtained data in table (3), fig (4) showed that the mean level concentrations of Pb, Cd, Cu, Zn in insects' tissues which were collected from loc.#1 was reported as; 0.06,0.03,0.20, 0.07 (µg/g dry weight) whereas, it was reported as; 0.12,0.03,0.10,0.09 (µg/g dry weight); respectively, in loc.#2. The data in table (3) showed a significant difference between the mean concentrations of Pb, Cu, Zn in insect tissues in the two locations Lake Manzala (spring, 2018). The results in the above-cited table found that Cu has the highest concentration level in the insect as 0.20 (µg/g

dry weight) in loc.#1 in Lake Manzala then Pb in loc.#2 as 0.12 (µg/g dry weight) followed by Zn as 0.09 (µg/g dry weight) in loc.#2. The arrangement of elements in the insect tissue was as; Cu > Pb > Zn > Cd. The present data represented in table (3), fig. (4), showed that the mean concentration of heavy metal in the insect tissues was significantly (p<0.001) different in beetles collected from the two sites of Lake Manzala.

Table (4): The mean concentration of activity level of ALT, AST and the mean concentration levels of the total protein content in insects collected from Lake Manzala, Spring, 2018.

	Loc.#1	Loc.#2	t-test	P
ALT (U/mg protein)	37±2.1*	42.6±1.8*	4.484*	0.002*
AST (U/mg protein)	50.8±1.3*	59±0.7*	12.362*	<0.001*
T.P. (mg/g tissue)	4.9±0.3*	4.02±0.4*	4.274*	0.003*

Data were expressed by using mean ± S.E. Statistically significant at p* ≤ 0.05

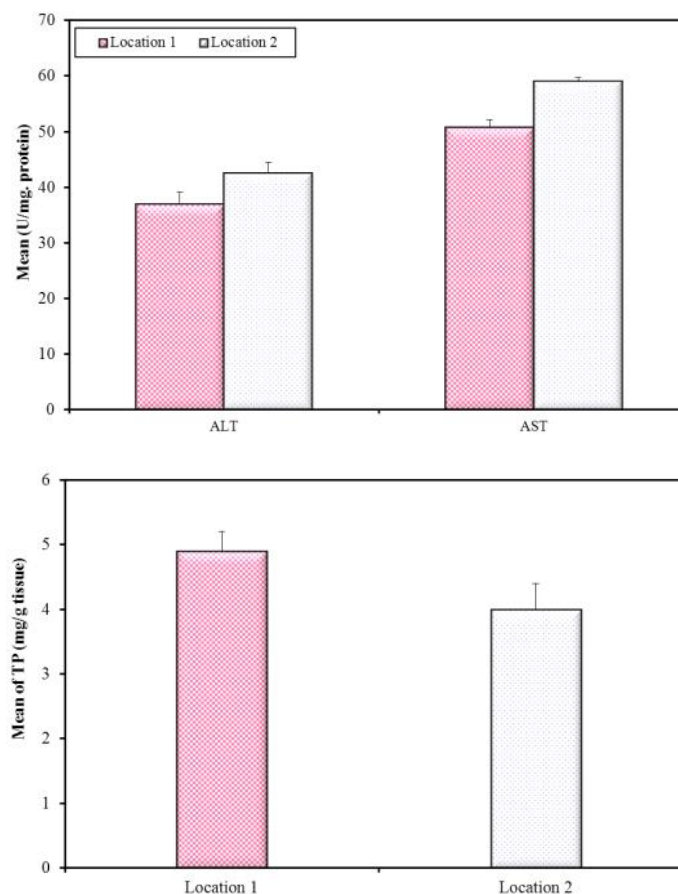


Fig. (5 a, b): Represents ALT, AST, T.P. collected from the insect in Lake Manzala, Spring, 2018

It is indicated from table (4), fig. (5), that there was a significant increase in the mean concentrations level of ALT activity in beetles in loc.# 2; 42.6 (U/mg protein), while it was 37 (U/mg protein) in that of loc.#1. The mean concentration level of AST activity in beetles in loc.#1 was 50.8 (U/mg protein), while it was 59 (U/mg protein) in that of loc.#2. The present results showed that there was a significant increase in the AST activity in samples collected from loc.#2 compared with that of loc.#1. The data cited in table

(4) indicated a significant decrease of the mean concentration level of the total protein content in beetles in loc.#2 compared with that of loc.#1. The level of total protein content was 4.9 (mg/g tissue) in loc.#1, while it was 4.02 (mg/g tissue) in that of loc.2. The mean concentration level of ALT and AST showed were high in insects collected from loc.#2 and the total protein was high in insects collected from loc.#1.

Table (5): Mean concentrations (mean ± S.D.) of MDA, GPx activities of beetles from Lake Manzala (loc.#1 and loc.#2)

	Loc.#1	Loc.#2	t-test	P
MDA (n mol/mg tissue)	14.02±0.4*	20.08±0.8*	15.112*	<0.001*
GPx (mU/mg. protein)	77.4±3.6	68.74±2.0*	4.735*	0.001*

Data were expressed by using mean ± S.E. Statistically significant at $p^* \leq 0.05$

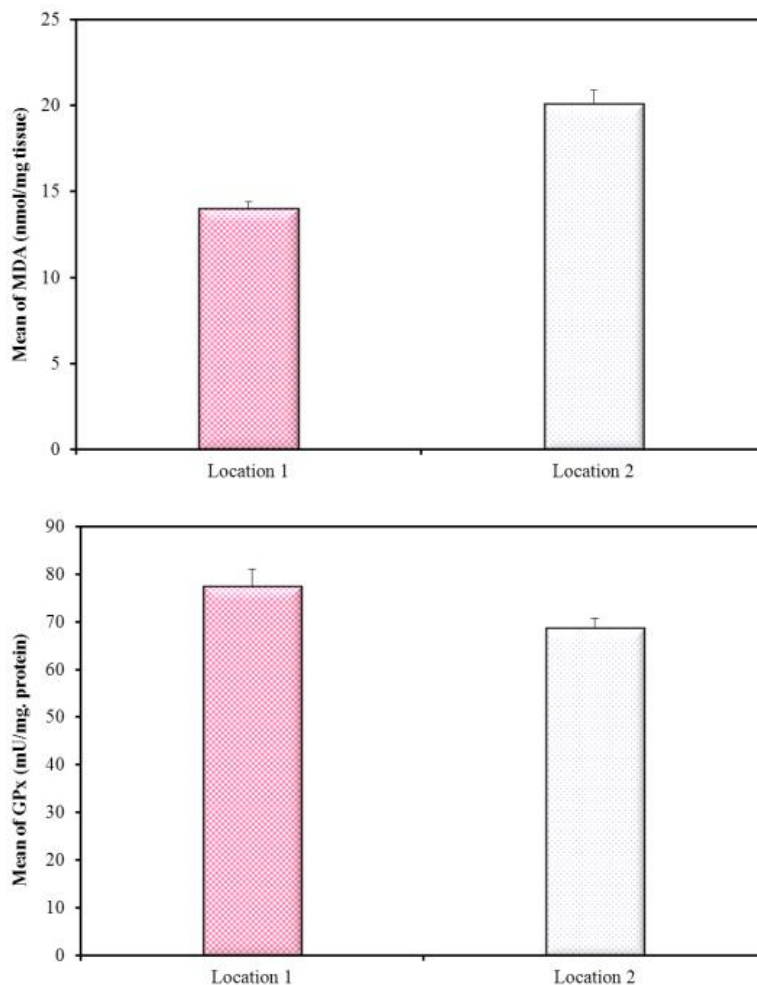


Fig. (6 a, b): Represents the mean concentration level of both GPx and MDA in insects collected from Lake Manzala, in Spring, 2018.

Table (5), fig.(6 a, b) shows the effects of heavy metals on the activity of the mean concentrations level of MDA in beetles tissues collected from Lake Manzala in spring, 2018. The data cited in the above table showed that the mean concentration level of MDA in loc.#1 was 14.02 (nmol/mg tissue), while it was 20.08 (nmol/mg tissue) in that of loc.#2 with a significant difference in concentration of MDA in the two locations. The data in

a table (5) showed a significant decrease in GPx activity in beetles in loc.#2 was 68.74 (mU/mg protein), while it was 77.4 in that of loc.#1. The results of the present study showed that there was a significant decrease in GPx activity in samples collected from loc.#2 compared with that of loc.#1 telling that loc.#2 is under stress of sewage pollution (Bahr El Baqur) drain.

Table (6): Correlation between different parameters in location 1

		Heavy metals in the water of the lake				Physicochemical parameters		Heavy metals in insect tissues				Biochemical parameters in insects			Effect of heavy metal pollution on some enzymes in insects	
		Pb (µg/l)	Cd (µg/l)	Cu (µg/l)	Zn (µg/l)	pH	ALK	Pb (µg/g)	Cd (µg/g)	Cu (µg/g)	Zn (µg/g)	ALT (U/mg. protein)	AST (U/mg. protein)	TP (mg/g tissue)	MDA (nmol/mg tissue)	GPx (mU/mg protein)
Pb (µg/l)	r	1.00	0.913*	-0.460	-0.786	0.496	-0.327	-0.757	-0.578	-0.262	-0.918*	-0.625	-0.415	-0.872	-0.867	-0.924*
	p		0.030*	0.436	0.115	0.395	0.592	0.138	0.308	0.671	0.028*	0.259	0.487	0.054	0.057	0.025
Cd (µg/l)	r		1.000	-0.072	-0.955*	0.444	-0.034	-0.933*	-0.389	-0.326	-0.771	-0.493	-0.363	-0.913*	-0.891*	-0.757
	p			0.908	0.011	0.453	0.957	0.021	0.518	0.592	0.127	0.399	0.548	0.030	0.043	0.138
Cu (µg/l)	r			1.000	-0.175	-0.203	0.603	-0.142	0.738	-0.219	0.552	0.333	0.407	0.258	0.292	0.666
	p				0.779	0.743	0.282	0.820	0.154	0.723	0.334	0.584	0.497	0.675	0.634	0.220
Zn (µg/l)	r				1.000	-0.520	0.017	0.894*	0.167	0.331	0.583	0.534	0.267	0.788	0.735	0.536
	p					0.369	0.978	0.040*	0.788	0.587	0.303	0.354	0.665	0.113	0.157	0.351
pH	r					1.000	-0.726	-0.123	-0.325	0.374	-0.161	-0.904*	-0.559	-0.350	-0.197	-0.243
	p						0.165	0.843	0.594	0.535	0.796	0.035	0.327	0.564	0.751	0.693
ALK	r						1.000	-0.311	0.232	-0.218	0.217	0.848	0.200	-0.061	-0.133	0.229
	p							0.610	0.707	0.724	0.726	0.069	0.747	0.923	0.831	0.712
Pb (µg/g)	r							1.000	0.227	0.484	0.693	0.172	0.179	0.854	0.873	0.644
	p								0.714	0.409	0.195	0.782	0.773	0.065	0.053	0.241
Cd (µg/g)	r								1.000	-0.494	0.495	0.203	0.870	0.690	0.666	0.737
	p									0.398	0.397	0.743	0.055	0.197	0.220	0.155
Cu (µg/g)	r									1.000	0.483	-0.020	-0.739	0.052	0.173	0.202
	P										0.410	0.975	0.154	0.934	0.781	0.744
Zn (µg/g)	r										1.000	0.408	0.161	0.742	0.810	0.951*
	p											0.496	0.796	0.151	0.097	0.013
ALT (U/mg. protein)	r											1.000	0.271	0.301	0.202	0.384
	p												0.659	0.622	0.745	0.524
AST (U/mg. protein)	r												1.000	0.630	0.526	0.442
	p													0.255	0.363	0.456
TP (mg/g tissue)	r													1.000	0.983*	0.840
	p														0.003*	0.075
MDA (nmol/mg tissue)	r														1.000	0.882*
	p															0.048
GPx (mU/mg. protein)	r															1.000
	p															

r: Pearson coefficient *: Statistically significant at $p \leq 0.05$

Table (6) in location #1 reported that, there are high negative correlations between Pb in water and Cd in water, Zn in water, Pb in insect tissue, Zn in insect tissues, ALT, TP, MDA, GPx, as $r=-0.913^*$, $r=-0.786$, $r=-0.757$, $r=-0.578$, $r=-0.918^*$, $r=-0.625$, $r=-0.872$, $r=-0.867$ and $r=-0.924^*$; respectively. Cd in water was negatively correlated with Zn in water, Pb in insect tissues, Zn in insect tissues, TP, MDA, GPx as $r=-0.955^*$, $r=-0.933^*$, $r=-0.771$, $r=-0.913^*$, $r=-0.891^*$, $r=-0.757$; respectively. Cu in water is positively correlated with ALK, Cd in insect tissues, Zn in insect tissues, GPx as $r=0.603$, $r=0.738$, $r=0.552$, and $r=0.666$; respectively.

Zn in water is positively correlated with Pb in insect tissues, TP, and MDA as $r=0.894^*$, $r=0.788$, $r=0.735$; respectively. The pH of water

in Lake Manzala is negatively correlated with, ALK, ALT, AST as $r=-0.726$, $r=-0.904^*$, and $r=-0.559$; respectively. ALK in the water of Lake Manzala in loc.#1 is positively correlated with ALT as $r=0.848$. Pb in insect tissues is positively correlated with Zn in insect tissues, TP, MDA, and GPx as $r=0.693$, $r=0.854$, $r=0.873$, and $r=0.644$; respectively. Cd in insect tissues is positively correlated with AST, TP, MDA and GPx as $r=0.870$, $r=0.690$, $r=0.666$ and $r=0.737$; respectively. Cu in insect tissues is negatively correlated with AST as $r=-0.739$. Zn in insect tissues is positively correlated with TP, MDA, and GPx as $r=0.742$, $r=0.810$, $r=0.951^*$; respectively. TP is highly positively correlated with MDA and GPx as $r=0.983^*$, and $r=0.840^*$; respectively. MDA is highly positively correlated with GPx as $r=0.882^*$.

Table (7): Correlation between different parameters in Location 2

		Heavy metals in the water of the lake				Physicochemical parameters		Heavy metals in insect tissues				Biochemical parameters in insects			Effect of heavy metal pollution on some enzymes in insects	
		Pb (µg/l)	Cd (µg/l)	Cu (µg/l)	Zn (µg/l)	pH	ALK	Pb (µg/g)	Cd (µg/g)	Cu (µg/g)	Zn (µg/g)	ALT (U/mg. protein)	AST (U/mg. protein)	TP (mg/g tissue)	MDA (nmol/mg tissue)	GPx (mU/mg. protein)
Pb (µg/l)	r	1.000	0.551	-0.405	-0.554	0.609	-0.280	0.070	-0.062	-0.456	-0.198	-0.188	0.577	-0.320	-0.008	-0.128
	p		0.335	0.499	0.333	0.276	0.649	0.911	0.922	0.441	0.750	0.763	0.308	0.599	0.990	0.837
Cd (µg/l)	r		1.000	0.117	-0.756	0.879*	-0.800	0.809	-0.114	-0.875	0.024	0.406	0.548	-0.962	-0.542	-0.825
	p			0.852	0.139	0.049	0.104	0.097	0.855	0.052	0.970	0.497	0.339	0.009	0.345	0.086
Cu (µg/l)	r			1.000	0.399	0.287	-0.653	0.651	0.755	-0.032	0.373	0.425	-0.321	-0.319	-0.885*	-0.044
	p				0.506	0.640	0.232	0.234	0.140	0.959	0.536	0.476	0.599	0.601	0.046	0.944
Zn (µg/l)	r				1.000	-0.659	0.251	-0.389	0.408	0.902*	-0.222	0.143	-0.287	0.618	-0.018	0.623
	p					0.226	0.684	0.517	0.495	0.037*	0.719	0.818	0.640	0.266	0.977	0.261
pH	r					1.000	-0.812	0.788	0.308	-0.876	0.364	0.087	0.224	-0.812	-0.674	-0.484
	p						0.095	0.113	0.614	0.051	0.547	0.889	0.718	0.095	0.213	0.409
ALK	r						1.000	-0.929*	-0.383	0.582	-0.094	-0.592	-0.341	0.872	0.923*	0.584
	p							0.023	0.525	0.303	0.880	0.293	0.575	0.054	0.025	0.301
Pb (µg/g)	r							1.000	0.277	-0.716	0.302	0.543	0.153	-0.916*	-0.878*	-0.704
	p								0.652	0.174	0.622	0.345	0.806	0.029	0.050	0.185
Cd (µg/g)	r								1.000	0.049	0.556	-0.176	-0.525	0.059	-0.655	0.484
	p									0.937	0.331	0.777	0.364	0.925	0.231	0.409
Cu (µg/g)	r									1.000	-0.436	0.000	-0.151	0.806	0.401	0.658
	p										0.463	1.000	0.809	0.099	0.504	0.228
Zn (µg/g)	r										1.000	-0.535	-0.813	-0.041	-0.309	0.193
	p											0.353	0.094	0.947	0.613	0.756
ALT (U/mg. protein)	r											1.000	0.584	-0.580	-0.490	-0.667
	p												0.301	0.305	0.402	0.219
AST (U/mg. protein)	r												1.000	-0.478	0.000	-0.576
	p													0.416	1.000	0.309
TP (mg/g tissue)	r													1.000	0.670	0.882*
	p														0.216	0.048
MDA (nmol/mg tissue)	r														1.000	0.330
	p															0.588
GPx (mU/mg. protein)	r															1.000
	p															

r: Pearson coefficient

*: Statistically significant at $p \leq 0.05$

Table (7) represents the correlations that are found in loc.#2 Lake Manzala. It is found here that Pb in water is positively correlated with pH and AST as $r=0.609$ and $r=0.577$; respectively. Cd in water is negatively correlated with Zn in water, ALK, Cu in insect tissues, TP, and GPx as $r=-0.756$, $r=-0.800$, $r=-0.875$, $r=-0.962$, and $r=-0.825$; respectively, while Cd in water is positively correlated with pH and Pb

in insect tissues as $r=0.879^*$ and $r=0.809$; respectively. Cu in water in loc.#2 is negatively correlated with ALK and MDA as $r=-0.633$ and $r=-0.885^*$; respectively, whereas, Cd in water is positively correlated with Pb in insect tissues as $r=0.755$. Zn in water is negatively correlated with pH as $r=-0.659$ while it is positively correlated with Cu

in insect tissues, TP, and GPx as $r=0.902^*$, $r=0.618$, and $r=0.623$; respectively.

The pH is positively correlated with Pb in insect tissues as $r=0.788$ whereas, it is negatively correlated with ALK, Cu in insect tissues, TP, MDA as $r=-0.812$, $r=-0.876$, $r=-0.812$, and $r=-0.674$; respectively. ALK is highly negatively correlated with Pb in insect tissues as $r=-0.929$ and with ALT as $r=-0.592$, whereas, it is positively correlated with Cu in insect tissues, TP, MDA, and GPx as $r=0.582$, $r=0.872$, $r=0.923^*$ and $r=0.584$; respectively. Pb in insect tissues is negatively correlated with Cu in insect tissues, TP, MDA, and GPx as $r=-0.716$, $r=-0.916^*$, $r=-0.878^*$, and $r=-0.704$; respectively. Cd in insect tissues is negatively correlated with MDA as $r=-0.655$. Cu in insect tissues is positively correlated with TP and GPx as $r=0.806$ and $r=0.658$; respectively. Zn in insect tissues is negatively correlated with AST as $r=-0.813$. ALT is positively correlated with AST as $r=0.584$ and negatively correlated with TP and GPx as $r=-0.580$ and $r=-0.667$; respectively. AST is negatively correlated with GPx as $r=-0.576$. TP is negatively correlated with MDA and GPx as $r=0.670$ and $r=0.882^*$; respectively. MDA is positively correlated with GPx as $r=0.588$.

Discussion:

The poisonous synthetic compounds released into water get into natural pecking order from the earth, causing wellbeing anomalies (Li and Kong, 2009). The satisfactory furthest reaches of Lead, Cadmium, Copper, Zinc ought not to surpass (0.05, 0.01, 1, 5 mg/L; individually) for the ordinary nearness implies focuses in water as indicated by USEPA (2005). The portrayal of the physiological trustworthiness needs biomarkers of introduction to natural stressors. Biomarkers can be characterized as quantifiable adjustments at the physiological levels (Lionetto *et al.*, 2003). The human effect has been incredibly changed over from a profitable lake to eutrophic bowls (Hamza, 1999).

The results showed that the water of the two locations Lake Manzala were on the alkaline side, but there was a decrease in pH value in loc.#2 (Eastern side of the lake) as the wastewater effluents are flowing into Manzala Lake from nine drains (Elmorsi *et al.*, 2017). The pH value is affected by the sewage discharges (Nessim *et al.*, 2005). The pH value is a significant factor in limiting the threshold concentration (Abou Taleb *et al.*, 2004). The present study revealed low alkalinity in the studied locations

and that Manzala Lake is not heavy polluted although that, Manzala Lake is suffering from environmental changes (Elmorsi *et al.*, 2017), it is known that the water far away from pollution had low alkalinity (El Rais and El Sabrouti, 1994). Aquatic insects have been utilized as bio-indicators of pollution (Maron and Monteiro, 2002; Nummelin and Lodenius, 2007).

In the present study, the results showed that the mean Pb concentration in beetle tissue collected from Lake Manzala ranged from (0.06-0.12 $\mu\text{g/g}$), this is because of the ability of insects to accumulate heavy metals in their tissue. Cd means concentration level in beetles' tissue in the water of Manzala was reported as 0.03 $\mu\text{g/g}$ in both locations. Cadmium may accumulate in aquatic tissues and affect their physiological functions (Sehar *et al.*, 2014). Cd leads to a gradual extinction of the aquatic organisms in polluted waters (Sridhara *et al.*, 2008; Radwan *et al.*, 2012; Radwan *et al.*, 2014; Radwan, 2016; Radwan *et al.*, 2016; Radwan *et al.*, 2017; Radwan *et al.*, 2018). Cadmium pollution has elevated for decades due to industrial, agricultural, and municipal wastes (Ursinova and Hladikova, 2000).

In the current study, Cu and Zn concentration in beetles from Lake Manzala ranged from (0.2-0.1 $\mu\text{g/g}$, 0.07-0.09); respectively. The antioxidant systems have great potential to indicate the cellular responses to heavy metals (Cirillo and Cocchieri, 2012). In the current study, the obtained results showed a significant increase in MDA concentration in insects' tissues in Lake Manzala (loc.#2, 20.08 nmol/mg tissue). The generation of ROS is restrained by the cancer prevention agent chemicals action. The generation of AST and ALT is because of the quality of amino corrosive for the procedure of gluconeogenesis (Stohs and Bagchi, 2001; Migula and Laszczyca, 2004). Sewage prompts increment in the action of both AST and ALT (El Demerdash and Yousef, 2004; Amin and Hashem, 2012). The obtained result in the current study showed a significant decrease in protein content in insects' tissues in Lake Manzala (loc.#2, 4.02 mg/g tissue).

The diminishing in protein content is because of the breakdown of protein into amino acids (Bizhannia *et al.*, 2005; Li *et al.*, 2012). Contamination stress can diminish the measure of aggregate protein substance of bugs (Bream, 2003; El barky *et al.*, 2008). Cancer prevention agent compounds are imperative parameters for testing water for the toxicant's nearness. In the present study, the results showed a significant decrease in

GPx activity in beetles' tissues in Lake Manzala (loc.#2, 68.74 mu/mg protein). The decrease of the total protein may mirror the reduction in the enzymatic exercises of GPx. The diminishing in protein substance could be because of the breakdown of protein into amino acids (Yan *et al.*, 2007). It is deduced in the present examination that creepy crawlies could be utilized as bio-pointers of oceanic contamination and that, the water nature of Lake Manzala is inside the standard water quality criteria for the freshwater (USEPA, 2005) except for Cd. It is incorporated from the gotten information that the eastern piece of the lake is greatly influenced by the swage contamination than the western piece of the lake Manzala. The end in the present examination that, the pH estimation of water the lake was soluble which comes in concurrence with (Elmorsi *et al.*, 2017) on their work in Manzala Lake.

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