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Valuation of water pollution using enzymatic biomarkers in aquatic insects as bioindicators from El-Mansouriya stream, Dakahlia, Egypt

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Abstract

This investigation was aimed to evaluate water pollution with heavy metals using three biomarkers, as well as the study of the aquatic insects-seasons interaction. Three species of aquatic insects collected from El-Mansouriya stream (Dakahlia, Egypt), during the different seasons from spring 2014 to winter 2015. Water samples, sediments and whole body of the three aquatic insects were analyzed for heavy metal contents. As well as, two enzyme biomarkers, Acetylcholinesterase (AChE) and glutathione-S-transferase (GST) were used to monitor aquatic insects' response to water pollutants. Results showed various activities of the three biomarkers at the different studied seasons. Where, the concentration of selected heavy metals, in the whole body of the three insects, showed a relatively wide range for each metal analyzed. Also, higher values of metal pollution index (MPI) were attained in hotter seasons (spring and summer) and its decrements were observable during cooler seasons (autumn, winter), that provides a representative picture to the environmental state of environmental impacts on the aquatic ecosystem. While the heavy metals were found to be highly accumulated in selected aquatic insects body, as well as the variation of enzymes activity, thus both enzymes could be used as biomarker of water and sediment contamination.

Keywords: Bio-monitors, Glutathione-S-transferase (GST), Acetylcholinesterase (AChE), Metal pollution index (MPI), Fresh water quality, Seasonal variations, Aga.

Introduction

Fresh water is an essential resource sustaining human life and other organisms in the environment to keep the balance of nature. In Egypt, the River Nile used for various purposes like agriculture, industries, hydropower, and fisheries and recreational uses (Abdel-Hamid *et al.,* 1992). The environmental impacts in any aquatic ecosystems can be assessed and monitored by physical and chemical techniques and also by biological methods (Foil, 1998; Chae *et al*., 2000). The aquatic insects are the most diverse group of fresh water benthic macroinvertebrates, spending all or part of their life cycle within water, that can be used as very good indicators of water qualities (Arimoro & Ikomi, 2008; Popoola & Otalekor, 2011).

Total heavy metals concentrations in aquatic ecosystem can mirror the pollution status, as heavy metals are non-degradable and can accumulate in living tissue (Haiyan & Stuane, 2003; Iken *et al.*, 2003). Where, Lambou & Williams (1980) and Chaphekar (1991) reported the dangerous effect of heavy metals that persistent and accumulate in water, sediments and tissues of the living organisms, through bioconcentration (i.e. uptake from the ambient environment and biomagnification (i.e. uptake through food chain). Heavy metals normally occurring in nature are not harmful to the environment, but their presence at higher concentrations become toxic, and pollution in relation to their toxicity to aquatic organisms affect ultimate wellbeing of humans (Canli & Atli, 2003; Yayıntas *et al*., 2007).

Khaled (2004) found that sediment analysis, allows identification of contaminants which are absorbed by particulate matter that escape detection by water analysis. However, Mohamed & Mohamed (2005) reported that, sediments are important sinks for various pollutants like pesticides and heavy metals and also play a significant role in the remobilization of contaminants in aquatic ecosystems under favorable conditions and in interactions between water and sediment. The analysis of sediments frequently reflected the sources of trace metal in the aquatic environment because of its high accumulation rates, so sediment quality is a good indicator of pollution in water column, where it tends to concentrate the heavy metals and other organic pollutants (Ibrahim, 2007; Aderinola *et al*., 2012).

The elevated level of trace metals at different organs of microorganisms is used as an important tool or index of metal pollution in an aquatic ecosystem (Tarrio *et al*., 1991; Mendil *et al*., 2005). In addition, Ravera (2001) reported that if an environment receives metal pollutants, the organisms living in it could take up the pollutants from the water or/and food and concentrate it in their bodies. Also, Tayel *et al*. (2008) observed that increase of heavy metals accumulation in fish returned to the increase of the total dissolved metals in Nile water and sediments. Meanwhile, Mokhtar *et al*. (2009) explained that metal pollution index (MPI) provides a representative picture of the environmental state of any environmental impacts on the aquatic ecosystem. In different regions of Egypt, especially agricultural and industrial regions, it is necessary to detect the heavy metals, which are the most appropriate indicator of pollution, because of their stability in sediment and scarcity in neutral environments (Girgin *et al*., 2010).

Aquatic insects are typically monitored for pollution by heavy metals using chemical or biological assays, as chemical measurements of heavy metals in water don’t provide information about the bio availability of them. Insects constitute important links in metal transport chain between trophic levels, therefore trace metal concentration in insects have an important influence on the trace metal distribution in the biosphere (Lindqvist & Block, 1995). Moreover, aquatic insects were reported by several authors as efficient biomonitors to heavy metal contamination (Abd-Allah *et al*., 2000; Bream, 2001). According to Krantzberg & stokes (1989) the highest accumulated metals in aquatic insects occur in immature stage than adult as their growth was retarded and metals were accumulated to higher concentration.

The use of biochemical biomarkers to measure the exposure of organisms to chemicals has been extensively reviewed by McCarthy & Shugart (1990), Benson & DiGiulio (1992), Mayer *et al*. (1992) and Lagadic *et al*. (1994). Resistance to pesticides in invertebrates by detoxification is the only population-level response that has been directly correlated with enzyme biomarkers such as glutathione-S-transferase (GST) activity and acetylcholinesterase (AChE) activity, or other esterase activity (Lagadic *et al*., 1994; Gunning *et al*., 1997). Heavy metals that accumulate in aquatic invertebrates may lead to oxidative stress and a defensive mechanisms are found in many aquatic organisms include various antioxidant enzymes, as GST and AChE that possess detoxifying activities towards lipid hydroperoxides generated by organic pollutants as well as heavy metals (Farombi *et al*., 2007; Siwela *et al*., 2010).

AChE plays an important role in neurotransmission in both vertebrates and invertebrates, and its activity has been widely studied and employed as a biomarker in aquatic invertebrate and vertebrate species to detect exposure to chemicals in natural ecosystem (Pena-Lopis *et al*., 2003; Romeo *et al*., 2003; Lavado *et al*., 2006). Furthermore, activity inhibition was correlated with the Zn, Cu and Cd accumulation in digestive gland and gills of treated clams (Kamel *et al*., 2012). GST represents an important family of enzymes that catalysts the conjugation of various electrophilic compounds and exists in multiple forms and also known to be involved in insect resistance to organophosphorus insecticides (Terriere, 1984; Clark, 1989). Hence, AChE and GST measurements in aquatic macroinvertebrates could be used as a biomarker of susceptibility (Hynea & Maher, 2003).

The present study was conducted to estimation the water pollution of the selected Egyptian stream based on accumulation of heavy metals in water and sediment and also investigate the accumulation of heavy metals in the selected three aquatic insects. Moreover, to explore the use of GST and AChE as biomarkers for pollution in aquatic ecosystems.

Materials and Methods

Description of the studied area:

Aga is a medium-sized city in Dakahlia governorate, Egypt, it is famous for agricultural and few industrial activities and El-Mansouriya stream run throughout it with average mean depth of 2-3 meter and a width of 19 meter. The stream is a natural aquatic ecosystem that receives agricultural drainage and the effluents of some near factories. It is situated within the vicinity of agriculture area, while El Mansoura principal road along from one side and the study was conducted between Longitude 31°17'16.91", 31°16'38.17" east and Latitude 30° 55'49.74", 30°53'26.98" north.

Water sampling and heavy metal determination:

Water samples were collected, during the period from spring 2015 to winter 2016, from depth not less than 30 cm using a Van Dorn water sampler with capacity of 1.2 litters and were kept in cleaned stoppered plastic bottles for further examination. They were then treated with 5 ml of concentrated nitric acid (HNO_3) to one liter of each sample and preserved immediately in a refrigerator at approximately 4^{o}c for analysis (APHA, 2005). In the laboratory, heavy metals were determined after the digestion with HNO_3, by adding 20 ml of nitric acid to 500 ml of preserved sample in a beaker and boiled slowly to evaporate on a hot plate to reach the lowest volume, before precipitation occurred or until completed digestion. Digested sample was transferred to 100 ml volumetric flask, the wall of the beaker was washed by deionized water and added to the same volumetric flask, and then completes the volume to 100 ml. This volumetric flask was cooled and again completed with deionized water and mixed thoroughly. Atomic absorption spectrophotometer was used for measuring the optical density for each sample and heavy metal concentrations was expressed as ppm. The determined concentration levels of heavy metals in water were then compared with WHO standards (2004).

Soil (sediment) sampling and heavy metal analysis:

Sediment sample (500 g) was taken from where the aquatic insects were collected, during the same period. In laboratory, samples were oven-dried (70°C) until a constant weight was attained, ground to a homogenous powder, and preserved in polythene bags. Sediment digestion was based on the protocol described by Quevauviller *et al*. (1993) and Vercoutere *et al*. (1995), where soil samples (0.5 g) placed in a digesting flask and pre-digested with a 12 ml of 37% HCl: 65% HNO_3 (3:1) mixture for 24 h at room temperature. The suspension was then digested to near dryness on a thermostatically controlled hotplate at 90 °C in a fume cupboard, following which 2.5 ml of 37% HCl and 2.5 ml of 30% H_2O_2 were added to complete the digestion. The resultant mixture was heated again and then cooled to ambient temperature and the flask walls were washed with 10 ml of deionized water and then the suspension was filtered through Whatman filter paper (No. 41) in a volumetric flask, diluted to 50 mL, and stored in polyethylene bottles at 4°C for later analyses. Concentrations of heavy metals in the samples were determined by Atomic absorption spectrophotometer and expressed as ppm dry weight. The determined concentration levels of heavy metals in sediment were then compared with WHO (2004) standards.

Aquatic insect's collection, identification and heavy metal analysis:

Aquatic insects were collected, during the same period at the early hours of the day, and sampling techniques was due to Downing & Rigler (1984) and Barbour *et al*. (1999) protocols, by hand picking and kick/sweep method using aquatic dip and sweeping nets (45µm mesh). Immediately collected insects were sorted by naked eyes and some of them placed in plastic labeled vials with 70% ethyl alcohol, then taken to taxonomy laboratory for identification using taxonomic keys (Badawy *et al*.,; 2013Degabriele, 2013). While the bulk of selected insects is freezed for heavy metal analysis and biochemical studies. The whole insect of each species was pre-weighed for 0.5 gm, using Electric balance (4 digital), freezed at -4˚C for 24 hours. Concentrated Nitric acid (Analar) (0.5-1ml) was added to each 25 mg tissue of each species, heated gently in the heating block (90˚C) and vortexed to help the tissue solublization. The dissolved tissue was then returned to the heating block till the color starts to turn brown, cooled and then 0.1 ml concentrated nitric acid was added for digestion. Thereafter, the digested tissue was returned back to the heating block till the volume was reduced to 0.25-0.5 ml, cooled, 0.1 ml H_2O_2 (30%) was added and then the dissolved tissue heated again in the heating block till the volume was reduced to 0.25-0.5 ml. Finally, the digested tissue was diluted with distilled water to 2 ml and analyzed using GBC

Atomic absorption spectrophotometer Savanta A A and expressed as ppm dry weight.

Sample preparation for biochemical studies:

The whole body of each selected insects were weighed and then homogenized in a saline solution (1gm of tissue insect/1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m for 15 min. The supernatant was used directly or frozen until the use for the metabolite determination, where three replicates were used. Activity of Glutathione-S-transeferase (GST) was determined according to the method of Habig *et al*. (1974) and that of acetyl cholinesterase enzyme (AChE) was determined by the method adopted by Ellman *et al*. (1961).

Data analysis:

Statistical analyses use one way a nova between heavy metal elements during seasons were done using package SPSS 20.0 for Windows7. The metal pollution index (MPI) was used in order to compare the total content of heavy metals at locations studied as shown below, where Cf_n is the concentration (*µg/g fresh wt*.) for metal in the sample (Usero *et al*., 1996).

$$MPI = (Cf_1 \times Cf_2 \times Cf_3 \ _{\dots} \times Cf_n)^{1/n}$$

Results

Seasonal variation of heavy metal in water and sediment:

The results of heavy metals concentration in water samples from Dakahlia given in table (1), revealed that the highest pollution level was recorded in summer, while the lowest was in winter. However, Fe, Zn and Co were higher in spring, while Fe and Zn were lower in winter and Co was lower in summer. In general, Mn and Zn were not detected in winter and Cd was not recorded in spring and winter. Moreover, Cr was absent in all seasons. While the results of heavy metal analysis in sediments from Dakahlia presented in table (1), showed that most of the heavy metals were higher in summer and lower in winter and autumn. However, Fe and Cu were higher (12.4, 8.8 ppm) in autumn and spring, and lower (6.0, 2.7 ppm) in summer and autumn, respectively. The data analysis showed no significant differences between seasons for Fe, Mn, Zn and Cu in water, while Cd and Co showed significant differences among different seasons. Whereas, a significant difference of heavy metals in sediments recorded among seasons.

Table (1). Seasonal variation of heavy metals in water & sediment from Dakahlia throughout one year of study period from spring 2014 till winter 2015.

Heavy metals	Water/Sediment	Spring	summer	Autumn	Winter
Fe	**Water**	0.86±0.35 ^{a}	0.74±0.29 ^{a}	0.63±0.38 ^{a}	0.62±0.27 ^{a}
	Sediment	6.3±1.6 ^{a}	6.0±1.8 ^{a}	12.4±1.7 ^{b}	10.3±1.4 ^{b}
Mn	**Water**	0.060±0.02 ^{a}	0.063±0.21 ^{a}	0.05±0.26 ^{a}	--
	Sediment	6.04±1.1 ^{a}	6.0±1.0 ^{a}	2.9±0.34 ^{b}	2.0±0.8 ^{b}
Zn	**Water**	0.21±0.02 ^{a}	0.063±0.02 ^{a}	0.05±0.03 ^{a}	--
	Sediment	3.3±0.8 ^{a,b}	3.9±1.3 ^{b}	1.9±0.6 ^{a}	2.7±0.9 ^{a,b}
Cu	**Water**	0.25±0.06 ^{a}	0.59±0.19 ^{b}	0.13±0.06 ^{a}	0.09±0.02 ^{a}
	Sediment	8.8±1.4 ^{a}	6.8±2.1 ^{a,c}	2.7±0.7 ^{b,d}	4.4±1.1 ^{c,d}
Cd	**Water**	--	0.01±0.002 ^{a}	0.01±0.001 ^{a}	--
	Sediment	3.42±1.0 ^{a}	3.7±1.1 ^{a}	1.1±1.4 ^{b}	1.5±0.4 ^{b}
Cr	**Water**	--	--	--	--
	Sediment	3.5±0.8 ^{a}	3.6±0.6 ^{a}	2.9±1.1 ^{a,b}	1.7±0.5 ^{b}
Co	**Water**	0.08±0.02 ^{a}	0.05±0.01 ^{b}	0.07±0.02^{a,b}	0.06±0.02 ^{a,b}
	Sediment	2.2±0.4 ^{a}	4.0±1.2 ^{b}	2.2±0.3 ^{a}	1.8±0.8 ^{a}

(a): Not significantly different ($P>0.05$), (b): Significantly different ($P<0.05$), (c): Highly significantly different ($P<0.01$), (d): Very highly significantly different ($P<0.001$)., --:not detected.

Heavy metals accumulation in insect tissues:

The results of heavy metals accumulated in *Crocothemis* sp. collected from Dakahlia are given in table (2). The highest concentration levels of heavy metals detected during summer and spring, while it decreased during winter. Generally, the average concentration level of Fe found to be the premier between metals regardless the season inspected. The concentrations of Fe, Cu and Cr exhibited a wide range of variation among different seasons, where in summer their concentration levels were 264.8, 19.9 and 10.1 ppm, while in winter were 97.5, 4.2 and 2.5 ppm. The results prevailed significant differences between metals and seasons, especially in the case of Cu. The highest concentrations of Mn, Zn and Co were recorded in spring (101.9, 35.4 and 22 ppm), while they reached their minimum values during winter (50.7, 25.8 and 10 ppm). An obvious significant difference, between these metals and seasons recorded mainly in the case of Mn. The highest concentration of Cd was recorded during autumn (80.6 ppm) and lowering during winter months (17.3 ppm), where the values found to be slightly varies significantly among seasons. The results revealed that MPI values were increased during summer season and lowering during winter.

Table (2): Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Crocothemis sp.* collected from Dakahlia during the period from spring 2014 till winter 2015.

Heavy metals	Spring	Summer	Autumn	Winter	Conc. levels Mean
Fe	239.6±6.02 ^{b}	264.8±7.11 ^{a}	104.1±7.45 ^{c}	97.5±2.52 ^{c}	176.5±88.1
Mn	101.9±4.51 ^{b}	93.9±3.62 ^{a}	66.01±5.12 ^{c}	50.7±2.30 ^{d}	78.1± 23.9
Zn	35.4±2.02 ^{b}	29.2±2.3 ^{a,c}	31.6±1.$^{5\ a}$	25.8±1.4 ^{c}	30.5± 4.0
Cu	13.6±2.4 ^{b}	19.9±2.1 ^{a}	9.4±1.7 ^{c}	4.2±1.01 ^{d}	11.8± 6.6
Cd	20.6±1.9 ^{b}	72.3±7.13 ^{a}	80.6±3.01 ^{c}	17.3±2.1 ^{b}	47.7± 33.4
Cr	9.7±1.8 ^{a}	10.1±1.2 ^{a}	8.4±0.9 ^{a}	2.5±0.72 ^{b}	7.7± 3.5
Co	22±2.6 ^{a}	20.4±1.9 ^{a}	19.4±0.6 ^{a}	10.0±1.2 ^{b}	18.0± 5.4
MPI	34	41.7	16.6	15.7	27.0 ± 12.9

a, b, c, d : See footnote of table (1), MPI: metal pollution index.

The results of heavy metals accumulated in *Ischnura* sp. collected from Dakahlia are presented in table (3). Totally, Fe concentration levels found to be the largest between metals regardless the seasons inspected. The highest concentration levels of metals were detected during summer except for Mn and Zn, where they reached their maximum values during spring. The metals decreased during autumn except Zn which lowering during summer. The concentration of Fe, Cu, Cd, Cr and Co exhibited a wide range of variation among different seasons, where their maximum values during summer found to be 184, 9.6, 105.4, 6.4 and 12.9 ppm, respectively. While they attend their minimum values during autumn (105.3, 3.3, 67, 2.9 and 2.8 ppm). The statistical analysis proved that the relationships between the aforementioned metals and seasons are significant, particularly in the case of Cd. However, the highest concentration of Mn and Zn were recorded in spring (83.5 and 31.2 ppm), while they reached their lowest values during autumn and summer (44.4 and 20.5 ppm). An obvious significant difference, between metals and seasons were recorded mainly in the case of Mn. The results offered in the below mentioned table cleared that MPI values were high in summer and low in autumn.

Table (3): Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Ischnura* sp. collected from Dakahlia during the period from spring 2014 till winter 2015.

Heavy metals	Spring	Summer	Autumn	Winter	Conc. levels Mean
Fe	177.9±2.0 ^{a}	184.0±4.2 ^{a}	105.3±5.5 ^{b}	….	155.7±43.8
Mn	83.5±2.9 ^{b}	75.3±2.1 ^{a}	44.4±1.4 ^{c}	….	67.7 ± 20.6
Zn	31.2±2.5 ^{b}	20.5±2.0 ^{a}	27.4±1.4 ^{b}	….	26.4 ± 5.4
Cu	5.4±1.2 ^{b}	9.6±1.9 ^{a}	3.3±1.3 ^{b}	….	6.1± 3.2
Cd	94.2±3.9 ^{c}	105.4±5.4 ^{a}	67.0±4.1 ^{b}	….	88.9 ± 19.7
Cr	3.1±1.2 ^{b}	6.4±1.01 ^{a}	2.9±0.7 ^{b}	….	4.1 ± 1.9
Co	11.3±0.9 ^{a}	12.9±1.5 ^{a}	2.8±1.5 ^{b}	….	9.0 ± 5.4
MPI	24.7	30.5	16.6	….	23.9± 7.0

a, b, c, d: See footnote of table (1). MPI: See footnote of table (2).

Data given in table (4) showed the heavy metals accumulated in *Diplonychus urinator (Durfour)* collected from Dakahlia. Entirely, the average concentration level of Fe found to be the major between metals regardless the seasons checked. The highest concentration levels of heavy metals were detected during summer and spring, except Cd that attained its maximum value during autumn. The highest concentration levels of Fe, Cr and Cu were recorded in summer (372.2, 2.5 and15.5 ppm). Where the lowest values for Fe and Cr were detected during autumn (298.2 and 1.7 ppm) and that of Cu was inspected during spring (11.3 ppm).These results clarified significant differences among metals and seasons, mainly in the case of Fe. The maximum values for Mn, Zn and Co were recorded in spring (76.4, 30.1 and 7.1 ppm), while they reached their lowest values during winter (43.5, 10.2 and 2.6 ppm). Obvious significant differences, between metals and seasons were recorded principally in the case of Mn. The increments in MPI values were attained in summer and its decrements were observable during autumn.

Table (4): Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Diplonychus urinator* (Durfour) (=*Sphaerodema urinator*) collected from Dakahlia during the period from spring 2014 till winter 2015.

Heavy metals	Spring	Summer	Autumn	Winter	Conc. levels Mean
Fe	310.3±4.1 ^{b}	373.4±3.0 ^{c}	298.2±4.1 ^{d}	272.2±4.$^{4\ a}$	313.5±42.0
Mn	76.4±4.1 ^{b}	63.5±3.1 ^{a}	50.8±3.2 ^{c}	43.5±3.5 ^{d}	58.6 ± 14.5
Zn	30.1±1.7 ^{b}	22.2±2.0 ^{a}	22.1±1.9 ^{a}	10.2±1.6 ^{c}	21.2 ± 8.2
Cu	11.3±1.8 ^{b}	15.5±1.5 ^{a}	12.6±2.4 ^{b}	13.9±2.4 ^{c}	13.3 ± 1.8
Cd	32.5±28.2 ^{b}	66.6±2.4 ^{a}	75.1±2.0 ^{a,b}	33.0±2.8 ^{a,b}	51.8 ± 22.3
Cr	2.2±0.6 ^{a}	2.5±0.5 ^{a}	1.7±0.7 ^{a}	2.1±1.0 ^{a}	2.1 ± 0.3
Co	7.1±1.7 ^{b}	4.2±1.1 ^{a}	3.9±1.6 ^{a}	2.6±1.1 ^{a}	4.5 ± 1.9
MPI	23.7	24.8	21.3	16.5	21.6 ± 3.7

a, b, c, d : See footnote of table (1). MPI: See footnote of table (2).

The activity of detoxification enzymes in insect tissues:

The results of the activity of Acetycholinesterase (AChE) in insects collected from Dakahlia summarized in table (5), showed that different insects' species as well as different seasons gave significantly different means, where *Diplonychus urinator* (Durfour) gave the highest mean in all seasons, whereas *Ischnura* sp. gave the lowest in autumn and winter. Additionally, the results also showed a seasonal variation in all tested biomarkers, with higher activity values in hotter seasons (spring and summer) for these insects except for *Crocothemis* sp.

Table (5): Activity of Acetycholinesterase enzyme ($\bar{x}$±SD u/mg) in whole body homogenates of sampling insects collected from Dakahlia during the period from spring 2014 till winter 2015.

Insect species	Acetycholinesterase enzyme ($\bar{x}$±SD u/mg)			
	Spring	Summer	Autumn	Winter
***Crocothemis* sp.**	2.5 ± 0.6 ^{a}	6.2 ± 0.6 ^{b}	2.0 ± 0.4 ^{a}	2.8 ± 0.8 ^{a}
***Ischnura* sp.**	3.4 ± 0.7 ^{a}	5.3 ± 1.0 ^{b}	1.4 ± 0.3 ^{c}	--
***Diplonychus urinator* (Durfour)**	19.8 ± 1.4 ^{a}	27.3 ± 1.8 ^{b}	15.3 ± 1.3 ^{c}	12.6 ± 1.1 ^{d}

a, b, c, d: See footnote of table (1).

Data given in table (6), showed the activity of Glutathione-S-transferase (GST), in the three insect species as well as different seasons gave significantly different means, where *Diplonychus urinator* (Durfour) gave the highest mean in all seasons. Whereas *Ischnura* sp. gave the lowest in spring and autumn, while *Crocothemis* sp. gave the lowest in autumn and winter. Additionally, the results also showed a seasonal variation in all tested biomarkers, with higher activity values in hotter seasons (spring and summer) for the three insects.

Table (6): Activity of Glutathione-S-transferase enzyme ($\bar{x}$±SD u/mg) in whole body homogenates of sampling insects collected from Dakahlia during the period from spring 2014 till winter 2015.

Insect species	Glutathione-S-transferase enzyme ($\bar{x}$±SD u/mg)			
	Spring	Summer	Autumn	Winter
***Crocothemis* sp.**	13.4 ± 1.2 ^{a}	15.9 ± 1.6 ^{b}	5.6 ± 0.9 ^{c}	3.8 ± 0.8 ^{c}
***Ischnura* sp.**	7.7 ± 1.2 ^{a}	13.1 ± 0.9 ^{b}	6.3 ± 0.9 c	---
***Diplonychus urinator* (Durfour)**	37.6 ± 1.6 ^{a}	45.9 ± 2.3 ^{b}	23.8 ± 1.4 ^{c}	17.9 ± 1.2 ^{d}

a, b, c, d: See footnote of table (1).

Discussion

Seasonal variation of heavy metals in water:

An accordance to Haiyan & Stuane (2003) and Iken *et al.* (2003), total heavy metals concentrations in aquatic ecosystem can mirror the present pollution status as they are non-degradable and can accumulate in living tissue. In results, the order of heavy metals accumulation, detected in water in Dakahlia, were as follows: Fe > Cu > Mn > Zn > Co > Cd.

Iron (Fe) as clarified is the highest trace metal, because it is an abundant and important element, unsurpassed by any other heavy metals in the earth's crust (El-Naggar *et al.*, 2009). Although its concentration levels were generally within permissible limits of WHO (2004). Zinc (Zn) is an essential element for living organisms as it is crucial in protein metabolism since it is required for the correct functioning of many proteases (Vallee & Falchuk, 1993). Although Zn is an essential trace element, its high levels can cause harmful health effects and its toxicity to man is well known (Clark *et al.*, 1981). According to Wood (1974) and Aprile & Bouvy (2008), Zn is one of the most toxic and relatively accessible metal which when exposed to the atmosphere and or washed to the open waters will ultimately contribute to the pollution loading within the area. In this study, Zn concentrations are within permissible limits of WHO (2004) and actually in it lower range that confirm the acceptable industrial sewage. Cadmium (Cd) is one of the most toxic heavy metals and is considered as non-essential for living organisms (Woodbury, 1998). Although Cd was absent in some seasons, it was also recorded equal to the upper permissible level according to WHO (2004). Its absence may be due to the great tendency of Cd to be adsorbed on the suspended matter, while its high level may be due to its uptake and release by phyto- and zooplankton and other organisms in water, or the cycling of Cd from lower depths to the surface by plants (Laxen, 1985; Harrison & De Mora, 1996).

Manganese (Mn) is one of the most abundant among trace elements in the lithosphere (Torri *et al*. 2011). Although the level of Mn in water was within the permissible level according to WHO (2004) and this result may be attributed to low solubility of manganese in water or may be lowest in a more oxidizing condition (Osman & Kloas, 2010). Meanwhile, Cobalt (Co) is considered as an essential element, which is required in the normal human diet in the form of vitamin B12, cyanocobalamin (Gil *et al.*, 2008). However, the ingestion or inhalation of large doses of this trace element may lead to toxic effects, but Co is not withstanding in water as rocks are associated with Co to be slowly weathered and dissolute (Meck *et al.*, 2010). In present study, Co concentration was slightly above the permissible level of WHO (2004).

These seasonal variations may be due to the fluctuation in the amount of agricultural drainage water, sewage effluents and industrial wastes discharged into the lake (Zyadah, 1995). Increasing of metal concentrations in the water during hot seasons (spring and summer) detected in the current results may be attributed to the lease of heavy metals from the sediment to the overlying water under the effect of both high temperature and a fermentation process resulting from the decomposition of organic matter, as mentioned by Ali & Abdel-Satar (2005).

Seasonal variation of heavy metals in sediment:

Sediments are sinks for pollutants and so its quality is a good indicator of pollution in water column (Mohamed & Mohamed, 2005). It isn't strange that the accumulation of heavy metals in sediment detected was more than in water that agreed with the observation of Chindah & Braide (2003) and Eja *et al.*, (2003). The current analysis of heavy metals concentrations detected in sediment, cleared that their accumulation order as follows: Fe > Co > Cu > Mn > Zn > Cr > Cd.

As agreement with water analysis; iron (Fe) is the highest trace metal because it is an abundant and important element for oxygen transport and cellular respiration in all animals (Dojlido & Best, 1993). Although, the concentration level of Fe increased slightly during cooler seasons, it was still within the lower range of the permissible level of WHO (2004). Although, the concentration level of Mn detected in this study increased slightly during hotter seasons, it was still within the lower range of the permissible level of WHO (2004). Furthermore, Sudha Rani & Manikya Reddy (2003), Khaled (2004) and Osman (2012) testified the enrichment of this element in particulate matter due to the domestic and industrial waste inputs resulted in low dissolved oxygen content with H_2S formation by bacterial. Zn and Cu concentrations of sediment samples detected in this stream were lower than permissible level of WHO (2004), and the lower concentrations of Zn might be due to leaching of heavy metals into the deeper layer of the soil and to the ground water (Gil-Díaz *et al.*, 2014). Moreover, the data showed the lowest level of Zn during autumn, which may be related to the behavior of iron, where the increase of Fe in this season supported the explanation that attributed the decrease of zinc concentration due to its adsorption on $Fe(OH)_3$ sedimentation (Martinez *et al.*, 2000). On the other hand, Hong-yun *et al.*, (2005) look forwarded to the presence of copper in sediment due to mining, smelting, application of fertilizers and sewage sludge, along with the use of fungicides containing Cu and other human activities that led to widespread soil contamination with Cu, which is not detected in this study of selected stream excluding this reason for increasing pollution.

Cadmium (Cd) occurs naturally in soil as a result of the weathering of the parent rock, but most natural soil contain less than 1 mg kg^{-1} cadmium from the weathering of parent materials, those developed on black shales and those associated with mineralized deposits can have much higher levels (Alloway, 1995). However, the concentrations of Cd surpassed WHO (2004) values in sediment samples collected from current study especially hotter seasons, where this was probably due to the uses of phosphatic fertilizers, the cycling of Cd from lower depths to the surface by plants, and atmospheric deposition (Alloway, 1995; ATSDR, 2008). So, anthropogenic sources of Cd are highly significant than natural emissions and account for its ubiquitous presence in soil (Alloway, 1995; ECB, 2007). As known about chromium, it is one of the less common elements that does not occur naturally in elemental form, but only in compounds and usually found to be lowest in a more oxidizing condition (Osman & Kloas, 2010; Dotaniya *et al*., 2014). Unlike the results of heavy metal in water, lower range of permissible level of WHO (2004) is detected in sediment. Cobalt (Co) in the sampled sediments detected lower concentrations in comparison to the values of WHO (2004), unlike their concentrations in water, that may be attributed to the release of Co from the sediment into water.

More and more attention has been drawn due to the wide occurrence of metal pollution in aquatic system. Some heavy metals may transform into the persistent metallic compounds with high toxicity, which can be bioaccumulated in the organisms, magnified in the food chain, thus threatening human health (Zhou *et al*., 2008). Furthermore, Elewa & Goher (1999) illustrated the environmental factors which affected the precipitation of some heavy metals in Damietta Branch, and they indicated that the deposition of metals increased by the increase of pH, dissolved oxygen, organic matter and fine grain size of sediment.

Heavy metals accumulation in insects:

Routine chemical analysis that has been widely used to quantify the contaminants is not helpful and actually is a kind of troublesome (Abd-Allah & Bream, 2001). Therefore, employing the living organisms or what is referred to as biological monitors or "biomonitors" to monitor the contaminants is necessary. This method has advance on the other, not only to identify and quantify the already existed contaminants but also the newly added contaminants and can follow their spatial changes with the time and through the different months or seasons (Abd-Allah, 1999). Moreover, Aquatic insects were reported by several authors as efficient biomonitors to the contaminants of heavy metals (Abd-Allah *et al*., 2000; Bream, 2001). Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring them in water, sediments and biota, which generally exist in low levels in water and attain considerable concentration in sediments and biota (Rashed, 2001; Lasheen *et al*., 2012).

Generally, in our study the concentration of selected heavy metals, in whole body of selected insects, showed relatively wide range for each metal analyzed. Furthermore, Fe, Mn, Cd and Zn have the largest level, while Cr, and Co have the lowest one during the different seasons. This might reflect concentrations of these metals in water and sediment. Similarly, Tayel *et al*. (2008) observed that increase of heavy metals accumulation in fish, returned to the increase of total dissolved metals in Nile water and sediments. Also, Farombi *et al*. (2007) and Bahnasawy & Khidr (2011) found that the presence of higher concentration of metals in different fish species, due to exposure to higher concentration levels of these elements through water and sediments. Meanwhile, metal pollution index (MPI) provides a representative picture of the environmental state of any environmental impacts on the aquatic ecosystem (Mokhtar *et al*., 2009). Hence, in current results the increments in MPI values in selected stream were attained in hotter seasons (spring, summer) and its decrements were observable during cooler seasons (autumn, winter), that might be related to the increased metabolism of aquatic invertebrates (Khaled, 2004; Bahnasawy & Khidr, 2011).

Moreover, Cain *et al*. (1995) stated that the results of whole body metal concentration were higher and more accurate from another organ in insects. Hence, the present study, reported that highest accumulated metals in water, sediment and insects were Fe, Mn and Zn. This synchronization in results is in agreement with the findings of Shakweer (1998), who concluded that the concentration of trace metals in various organs of aquatic invertebrates reflects the degree of water pollution in the aquatic environments.

According to our investigation, *Diplonychus urinator* and *Crocothemis* sp. stored highest levels of lead, manganese and cadmium followed by *Ischnura* sp. which stored highest levels of lead, cadmium and manganese. Also, *Crocothemis* sp. had the highest MPI especially in hotter seasons. This agreed with Abd- Allah *et al*. (2000) results that showed higher accumulation of lead toxicity for *Ischnura* nymphs.

Heavy metal concentrations in the selected aquatic insects vary considerably among different seasons possibly reflecting its differences in water and sediments from which these insects were sampled, ecological needs, metabolism and feeding patterns (Mansour & Sidky, 2002). Therefore, bioaccumulation of metals in insects confirmed to be considered as an index of metal pollution in the aquatic ecosystem (Karadede-Akin & Unlü, 2007; Tawari-Fufeyin & Ekaye, 2007). While, highest accumulated metals in aquatic insects occurred during hotter seasons, where water temperature and acidity increase and these leading to increase in uptake source of food from surrounding environment (Livonen *et al*., 1992).

On our study, the variations in accumulated of heavy metals in selected aquatic insects were correlated with their growth increment in agreement with Lindqvist (1995). Meanwhile, the increase of MPI in insects may be due to concentrate in the active metabolic organs either for long-term storage or excretion. This observation agreed with Omar *et al.* (2014), who reported that the metabolically active tissues had high affinity to concentrate the greatest amount of most metals in their tissues. In contrast, the lowest MPI may be related to the high fat-content in tissues with low affinity to combine with metals, in addition to the low metabolic activity of aquatic invertebrates (Uluturhan & Kucuksezgin, 2007).

The activity of detoxification Enzyme:

Heavy metals that accumulate in aquatic invertebrates make catalyze reactions that generate oxygen reactive species (ROS) which lead to oxidative stress (Siwela *et al*., 2010). Defensive mechanisms to counteract with ROS are found in many aquatic organisms include various antioxidant enzymes, such as glutathione-s-transferase (GST) and acetylcholinesterase (AChE), which possess detoxifying activities towards lipid hydroperoxides generated by organic pollutants as well as heavy metals (Farombi *et al*., 2007). Also, aquatic insects, more accurately the early life stages, tend to be more sensitive to various chemical contaminants than later life stages (Buchwalter *et al*., 2004). Thus, the use of biochemical biomarkers to measure the exposure of organisms to chemical pollution has been extensively reviewed by McCarthy & Shugart (1990), Benson & DiGiulio (1992), Mayer *et al*. (1992) and Lagadic *et al*. (1994).

On our results, the activity of these two enzymes for aquatic insects was estimated and significant differences found among different seasons. Seasonal differences in the AChE and GST activity have been shown, which might be related to the seasonal changes in water temperature (Chitmanat *et al*., 2008).

AChE activity was found higher in *Diplonychus urinator* especially in hotter seasons, and decreasing during cooler seasons. While *Crocothemis* sp. showed a decrease activity of AChE followed by the lowest in *Ischnura* sp., especially in autumn. The increase in AChE activity may be due to heavy metals enhancement to accumulation of acetylcholine at the synapses, so that the post-synaptic membrane is in a state of permanent stimulation (Senthil Nathan *et al*., 2008; Begum *et al*., 2011). This agreed with Gill *et al*. (1991), who reported increased AChE activity in skeletal muscles and brain of a fish species exposed to Cd for 48 hours. Also, Zatta *et al*. (2002) observed increases in AChE activity of rats treated orally with Al.

While, the AChE inhibition may be also due to accumulation of heavy metals or pollution. Day & Scott (1990) found that greater depression in AChE activity in several invertebrate species can be an indicator of exposure to pesticides. Moreover, activity inhibition was correlated with Zn, Cu and Cd accumulation in digestive gland and gills of treated clams (Kamel *et al*., 2012). Furthermore, pesticides, carbamates and heavy metals are known by their capacity to inhibit *in vitro* or *in vivo*, AChE activity (Galloway *et al*., 2004; Banni *et al*., 2005). Likewise, Lavado *et al*. (2006) found that, when using AChE as neurotoxicity biomarker, AChE was strongly inhibited in the muscle of invertebrate sampled from a river highly polluted by organophosphorous, carbamates, and heavy metals. While, Flannagan *et al.* (1978) cited that, inhibition of AChE activity, in heads of stonefly nymphs, was linearly correlated with concentration in nymphs poisoned by fenitrothion. Day & Scott (1990) and Ibrahim *et al*., (1998) demonstrated similar results for stoneflies and chironomids exposed to several organophosphorus compounds.

Meanwhile, GST activity was found to be higher in *Diplonychus urinator*, especially in the hotter seasons that decreased during cooler seasons. While *Crocothemis* sp. and *Ischnura* sp. showed the lowest activity of GST in all seasons. The increase of GST activity in selected aquatic insects may be due to impacted environments by complex discharges of contaminants (Hamed *et al*., 2003).

Interestingly, the heavy metals were found to be highly accumulated in *Diplonychus urinator* as well as the high enzymes activity, thus both enzymes could be used as a biomarker of water and sediment contamination (Lee, 1988).

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References

Abd-Allah A.T, 1999. Accumulation of heavy metals in the freshwater prosobranch *Bellamia unicolor*. 10th International Symposium on Biomonitoring, Karlsruhe, Germany.

Abd-Allah A.T and Bream A.S, 2001. “Investigations on the efficiency of some freshwater invertebrates as biological monitors for heavy metal pollution”. J. Egypt. Acad. Soc. Environ. Develop., (D-Environ. Studies), 2 (2): 67 - 80.

Abd-Allah A.T, Bream A.S & El-Meadaway A.O, 2000. Toxicity of heavy metal interaction to some fresh water invertebrates. Al-Azhar Bull. Sci., 11 (1): 9 - 15.

Abdel-Hamid M.I, Shaaban-Dessouki S.A & Skulberg O.M, 1992. Water quality of the River Nile in Egypt: I. physical and chemical characteristics. Archiv fuer Hydrobiologie, Supplement AHBSA, 8: 90.

Aderinola O.J, Clarke E.O, Olarinmoye O.M, Kusemiju V. and Anatekhai M.A, 2012. Heavy metals in surface water, sediments, fish and perwinkles of Lagos Lagoon American-Eurasian. J Agric Environ Sci., 5 (5): 609 - 617.

Ali M. and Abdel-Satar A, 2005. Studies of some heavy metals in water, sediment, fish and fish diets in some fish farms in El-Fayoum province. Egypt J Aquat Res., 31 (2): 261 -273.

Alloway B.J, 1995. Cadmium. In: Alloway, B.J. (ed.). Heavy Metals in Soils (2nd edn). Blackie Academic & Professional, London, UK, 122 - 147.

APHA, 2005. Standard methods for the examination of water and wastewater. 19thEdn. American Public Health Association, 1015, Fifteen street, NW Washington, D.C. 20 R.C. 0015.

Aprile F.M and Bouvy M, 2008. Distribution and enrichment of heavy metals in sediments at the tapacura River Basin, Northeastern Brazil. Braz. J. Aquat. Sci. Technol., 12 (1): 1 - 8.

Arimoro F.O and Ikomi R.B, 2008. Response of macroinvertebrates to abattoir wastes and other anthropogenic activities in a municipal stream in the Niger Delta, Nigeria. Environmentalist 28: 85 - 98.

ATSDR, 2008. Draft toxicological profile for cadmium Atlanta: US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

Badawy R.M, El Hoseny I and Talal M, 2013. Biodiversity and seasonal fluctuation of aquatic and semiaquatic insects in Rashid stream, Kafr El Zayat (Gharbyia governorate). Egyptian Academic Journal of Biological Sciences, 6 (1): 47 - 66.

Bahnasawy M and Khidr A.N.D, 2011. Assessment of heavy metal concentrations in water, plankton, and fish of Lake Manzala, Egypt. Turk J Zool., 35 (2): 271 - 280.

Banni M, Jebali J, Daubeze M, Clerandau C, Guerbej H, Narbonne J.F and Boussetta H, 2005. Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers. Biomarkers, 10 (2- 3):105 - 116.

Barbour M.T, Gerritsen J, Snyder B.D and Stribling J.B, 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. US EPA; Office of Water; Washington, D.C.

Begum N, Sharma B and Pandey R.S, 2011. Toxicity potential and anti AChE activity of some plant extracts in *Musca domestica*. J. Biofertil. Biopestici, 2 (108): 6.

Benson W.H and DiGiulio R.T, 1992. Biomarkers in hazard assessments of contaminated sediments. In: Burton Jr., G.A. (Ed.), Sediment Toxicity Assessment. Lewis, Boca Raton, FL, USA, pp. 241-265.

Bouchard R.W, 2004. Guide to Aquatic macroinvertebrates of the Upper Midwest. Water Resources Center, University of Minnesota, St Paul, MN, 208 pp.

Bream A.S, 2001. Seasonal variation in heavy metal accumulation in three aquatic insect species. Al-Azhar Bull. Sci. Proceeding of the 4th Int. Sci. Conf. Cairo 27-29, 385-392.

Buchwalter D.B, Sandahl J.F, Jenkins J.J and Curtis L.R, 2004. Roles of uptake, biotransformation, and target site sensitivity in determining the differential toxicity of chlorpyrifos to second to fourth instar *Chironomous riparius* (Meigen). Aquatic Toxicology, 66: 149-157.

Cain D.J, Luom S.N and Axtmann E.V, 1995. Influence of gut content in immature aquatic insects on assessments of environmental metal contamination. Canadian Journal of Fisheries and Aquatic Science, 52 (12): 2736 - 2746.

Canli M and Atli G, 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, 121: 129 - 136.

Chae S.J, Purstela N., Johnson E., Derock E., Lawler S.P and Madigan J.E, 2000. Infection of aquatic insects with trematode metacercariae carrying *Ehrilichia risticii* the case of the Potomac house fever. Journal of Medical Entomology, 37: 619 - 625.

Chaphekar S.B, 1991. An overview on bioindicators. J. Env. Biol., 12: 163 - 168.

Chindah, A.C and Braide S.A, 2003. Cadmium and Lead concentrations in fish species of a brackish wetland/ upper Bonny Estuary, Niger Delta. J Nig Environ Soc., 1 (3): 399 - 405.

Chitmanat C, Prakobsin N, Chaibu P and Traichaiyaporn S, 2008. The Use of acetylcholinesterase inhibition in river snails (*Sinotaia ingallsiana*) to determine the pesticide contamination in the Upper Ping River. Int. J. Agri. Biol., 10: 658 - 60, 1814 - 9596.

Clark A.G, 1989. The comparative enzymology of the glutathione s-transferases from non-vertebrate organisms. Comp. Biochem. Physiol., 92B: 419 - 446.

Clark B.G, Harvey D.G and Humphrey D.J, 1981. Veterinary Toxicology, 2nd ed. London: 238.

Day K.E. and Scott I.M, 1990. Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. Aquatic Toxicology, 18 (2-3): 201 - 224.

Dojlido J.R and Best G.A, 1993. Chemistry of water and water pollution Elis, Hardwood Ltd Britain.

Dotaniya M.L, Thakur J.K, Meena V.D, Jajoria D.K and Gopal Rathor, 2014. Chromium pollution: A threat to enviroment - A review. Agri. Review, 35 (2): 153 - 157.

Degabriele G, 2013. An overview of the dragonflies and damselflies of the Maltese Islands (Central Mediterranean) (Odonata). Bulletin of the Entomological Society of Malta, 6: 5 - 127.

Downing J.A and Rigler E.H, 1984. A manual on methods for the assessment of secondary productivity of in fresh water, 2nd edn. IBP Handbook No. 17, Blackwell Scientific Publications, Oxford. p. 19 - 58.

ECB, 2007. European Union Risk Assessment Report. Cadmium oxide and cadmium metal, Part 1 – environment, 72. EUR 22919 EN. Luxembourg: Office for Official Publications of the European Communities.

Eja M.E, Ogri O.R.A and Arikpo G.E, 2003. Bioconcentration of heavy metals in surface sediments from the Great kwa Rivers Estuary, Calabar, South Eastern Nigeria. J Nig Environ Soc., 2: 247-256.

Elewa A.A and Goher M.M, 1999. Environmental factors affecting the precipitation and dissolution of Fe, Mn, Zn, Cu, Pb and Cd in River Nile at Damietta branch. Bull. Fac. Sci., Zagazig Univ., 21(2): 114 - 136.

Ellman G.L, Dlane K, Courtney V, Andres J.R and Deatherstone R.M, 1961. A new and rapid colourimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88 - 95.

El-Naggar A, Mahmoud S & Tayel S, 2009. Bioaccumulation of Some Heavy Metals and Histopathological Alterations in Liver of Oreochromis Niloticus in Relation to Water Quality at Different Localities along the River Nile, Egypt," World Journal of Fish and Marine Sciences, 1, (2): 105 - 114.

Farombi E.O, Adelowo O.A and Ajimoko Y.R, 2007. Biomarkers of oxidative stress and heavy metals levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Ogun River. Int J Environ Res Public Health., 4 (2): 158 - 165.

Flannagan J.F, Lockhart W.L, Cobb D.G and Metner D, 1978. Stonefly (*Plecoptera*) head cholinesterase as an indicator of exposure to fenitrothion. The Manitoba Entomologist, 12: 42 - 48.

Foil L.D, 1998. Tabanids as vectors of disease agents. Parasitology Today, 5: 88 - 96.

Galloway T.S, Brown R.J, Browne M.A, Dissanayake A, Lowe D, Jones M and Depledge M.H, 2004. A multibiomarker approach to ecosystem management. Environmental Science and Technology, 38: 1723 - 1731.

Gil R.A, Gasquez J.A, Olsina R, Martinez L.D and Cerutti S, 2008. Cloud point extraction for cobalt preconcentration with on-line phase separation in a knotted reactor followed by ETAAS determination in drinking waters. Talanta, 76: 669 - 673.

Gil-Díaz M, Ortiz L.T, Costa G, Alonso J, Rodríguez-Membibre M.L, Sánchez-Fortún S, Pérez-Sanz A, Martín M and Lobo M.C, 2014. Immobilization and Leaching of Pb and Zn in an Acidic Soil Treated with Zerovalent Iron Nanoparticles (nZVI): Physicochemical and Toxicological Analysis of Leachates, Water Air Soil Pollut 225, DOI 10.1007/s11270-014.

Gill T.S, Teware H and Pande J, 1991. *In vivo*, and *in vitro* effects of cadmium on selected enzymes in different organs of the fish *Barbus conchonius* Ham. (rosy barb). Comparative Biochemistry and Physiology Part C, 100: 501 - 505.

Girgin S, Kazancı N and Dügel M, 2010. Relationship between aquatic insects and heavy metals in an urban stream using multivariate techniques. Int. J. Environ. Sci. Tech., 7 (4): 653-664, 1735-1472.

Gunning R.V, Moores G.D and Devonshire A.L, 1997. Biochemical resistance detection in Helicoverpa armigera in Australia. *Recent Res.* Dev. Entomol., 1: 203 - 213.

Habig W.H, Pabst M.J and Jakoby W.B, 1974. Glutathion-stranseferases: the first enzymztic step in mercaptturic acid formation. J. Biol. Chem., 249 (22): 7130 - 7139.

Haiyan W and Stuane A. O, 2003. Heavy metal pollution in air-water-soil-plant system of Zhuzhou City, Human Province, China. Water, Air and Soil Pollution, 147: 79 - 107.

Hamed R.R, Farid N.M, Elowa Sh. E and Abdalla A.M, 2003. Glutathione related enzyme levels of freshwater fish as bioindicators of pollution. The Environmentalist, 23: 313 - 322.

Harrison M. R and De Mora J. D, 1996. Introductory chemistry for environmental science. Cambridge Univ. Press: 301.

Hong-yun P, Xiao-e Y and Sheng-ke T, 2005. Accumulation and ultrastructural distribution of copper in Elsholtzia splendens. Zhejiang Univ., Sc A, 6B: 311 - 318.

Hynea V. R and Maher A. W, 2003. Invertebrate biomarkers: links to toxicosis that predict population decline. Ecotoxicology and Environmental Safety, 54: 366 - 374.

Ibrahim, A.T. 2007**.** Distribution patterns of some heavy metals and pollution induced changes in some organs of three Nile fish species from Assiut, Egypt. Assiut Univ., Egypt.

Ibrahim H, Kheir R, Helmi S, Lewis J and Crane M, 1998. Effects of organophosphorus, carbamate, pyrethroid and organochlorine pesticides, and a heavy metal on survival and cholinesterase activity of *Chironomus riparius* Meigen. Bull. Environ. Contam. Toxicol., 60: 448 - 455.

Iken A, Egiebro N. O, and Nyavor K, 2003. Trace Elements in Water, Fish and Sediment from Tuskegee Lake, South Eastern, U. S. A. Air, Water and Soil Pollution, 149: 51 - 75.

Kamel N, Jebali J, Banni M, Ben-Khedher S, Chouba L and Boussetta H, 2012. Biochemical Responses and metals levels in *Ruditapes decussatus* after exposure to treated municipal effluents. Ecotoxicology and Environmental Safety, 82: 40v-46.

Karadede-Akin H and Unlü E, 2007. Heavy metal concentrations in water, sediment, fish and some benthic organisms from Tigris River, Turkey. Environ Monitor Assess. , 131 (1-3): 323 - 337.

Khaled A, 2004. Heavy metals concentrations in certain tissues of five commercially important fishes from El-Max bay, Alexandria, Egypt. Egypt J Aquat Biol Fish., 8 (1): 51 - 64.

Krantzberg G. and stokes P.M, 1989. Metal regulation, tolerance, and body burdens in the larvae of genus Chironomus. Canadian Journal of Fisheries and Aquatic Sciences, 46 (3): 389 - 398.

Lagadic L, Caquet T and Ramade F, 1994. The role of biomarkers in environmental assessment (5). Invertebrate populations and communities. Ecotoxicology, 3: 193 - 208.

Lambou V. W and Williams L. R, 1980. Biological monitoring of hazardous waters in aquatic systems. Second Interagency Workshop on in-Situ Water Sensing: Biological Sensors, 11 - 18. Pensacola Beach, Florida.

Lasheen M.R, Abdel-Gawad F.Kh, Alaneny A.A and Abd El bary H.M.H, 2012. Fish as Bio Indicators in Aquatic Environmental Pollution Assessment: A Case Study in Abu-Rawash Area, Egypt. World Applied Sciences Journal, 19 (2): 265 - 275.

Lavado R, Urena R, Martin-Skilton R, Torreblanca A, Del Ramo J, Raldua D and Porte C, 2006. The combined use of chemical and biochemical markers to assess water quality along the Ebro River. Environmental Pollution, 139 (2): 330 - 339.

Laxen D. P. H, 1985. Trace metal adsorption/ coprecipitation on hydrous ferric under realistic conditions: Role of humic substances. Water Res., 19: 1229 - 1236.

Lee R.F, 1988. Glutathione S-transferase in marine invertebrates from Langesundfjord. Marine Ecology Progress Series, 46: 33.

Lindqvist L, 1995. Influence of metal concentration in food on metal uptake and accumulation in sawfly larvae. Archives of Enviromental Contamination and Toxicology, 28 (3): 310 - 313.

Lindqvist L and Block M, 1995. Excretion of cadmium during moulting and metamorphosis in *Tenebrio molitor* (Coleoptera; Tenebrionidae). Comp. Biochem. Phys., C 111: 325 - 328.

Livonen P, Piepponen S and Verta M, 1992. Factors affecting trace-metal bioaccumulation in Finnish headwater lakes. Environmental Pollution, 78 (1-3): 87 - 93.

Mansour S and Sidky M, 2002. Ecotoxicological Studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. Food Chem.; 78(1): 1 5-22.

Martinez M.E, Sanchez S, Jimenez J.M, El Yousfi F and Munoz L, 2000. "Nitrogen and phosphorus removal from urban wastewater by the *microalga Scenedesmus obliquus*". Bioresource Technol., 73: 263 - 272.

Mayer F.L, Versteeg D.J, McKee M.J, Folmar L.C, Graney R.L, McCune D.C and Rattner B.A, 1992. Physiological and nonspecific biomarkers. In: Huggett, R.J., Kimerle, R.A., Mehrle Jr., P.M., Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis, Boca Raton, FL, pp. 5 - 85.

McCarthy J.F and Shugart L.R, 1990. Biological markers of environmental contamination. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis, Boca Raton, FL, USA, pp. 3 - 14.

Meck M.L, Atlhopheng J, Masamba W.R.L and Ringrose S, 2010. Pollution impliocations of save river water from weathering and dissolution of metal hosting minerals at Dorowa phosphate mine, Zimbabwe. Phys. Chem. Earth, 35: 679 - 685.

Mendil D, Uluozlu O.D, Hasdemir E, Tuzen M, Sari H and Suicmez M, 2005. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chemistry, 90: 175 - 179.

Mohamed H. H. A and Mohamed R. A. F, 2005. Accumulation of Trace Metals in Some Benthic Invertebrate and Fish Species Relevant to Their Concentration in Water and Sediment of Lake Qarun, Egypt. Egyptian Journal of Aquatic Research, (31)1: 289 - 301.

Mokhtar M.B, Aris A.Z, Munusamy V and Praveena S. M, 2009. Assessment level of heavy metals in Penaeus Monodonta and Oreochromis Spp in selected Aquaculture Ponds of High Densities Development area. European Journal of Scientific Research, 30 (3): 348 - 360.

Omar W.O, Saleh Y.S and Marie M.A.S, 2014. Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. Ecotoxicology and Environmental Safety, 110: 221 - 231.

Osman A and Kloas W, 2010. Water quality and heavy metal monitoring in water, sediments, and tissues of the African Catfish *Clarias gariepinus* (Burchell, 1 822) from the River Nile Egypt. J. Environ. Protect., 1: 389 - 400.

Osman A, 2012. Biomarkers in Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) to assess the impacts of River Nile pollution: bioaccumulation, biochemical and tissues biomarkers. J. Environ. Protect., 3: 966 - 977.

Pena-Lopis S, Dolores Ferrando M and Pena J.B, 2003. Fish tolerance to organoohosphate- induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcycteine. Aquatic Toxicology, 65 (4): 337 - 360.

Popoola K.O.K and Otalekor A, 2011. Analysis of Aquatic Insects' Communities of Awba Reservoir and its Physico-Chemical Properties. Research Journal of Environmental and Earth Sciences, 3(4): 422 - 428.

Quevauviller P, Imbert J and Olle M, 1993. Evaluation of the use of microwave oven systems for the digestion of environmental samples. Mikrochim. Acta., 112: 147 - 154.

Rashed M.N, 2001. Monitoring of environmental heavy metals in fish from Nasser lake. Environ. Int., 27: 27 of Spain. Environ. Int., 29: 949 - 956.

Ravera O, 2001. A comparison between diversity, Similarity, and biotic indices applied to the macroinvertebrate community of a small stream: The Ravella River (Como Province, N. Italy). Aquat. Ecol., 35: 97 - 107.

Romeo M, Hoarau P, Garello G, Gnassia-Barelli M and Girard J.P, 2003. Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environmental Pollution, 122 (3): 369 - 378.

Senthil Nathan S, Choi M.Y, Seo H.Y, Paik C.H, Kalaivani K and Kim J.D, 2008. Effect of azadirachtin on acetylcholinesterase (AchE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stal). Ecotoxicol. Environ. Saf., 70 (2): 244 - 250.

Shakweer L.M, 1998. Concentration levels of some trace metals in *Oreochromis niloticus* at highly and less polluted areas of Mariut Lake. J. Egypt Ger. Soc. Zool., 25: 125 - 1 44.

Siwela A.H, Nyathi C and Naik Y.S, 2010. A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaea natalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe. Ecotoxicol. Environ. Saf., 73: 1728 - 1732.

Sudha Rani P and Manikya Reddy P, 2003. Preliminary studies on metal concentration on Hussain Sagar Lake. Poll. Res., 22 (3): 377 - 380.

Tarrio J, Jaffor M and Ashraf M, 1991. Levels of selected heavy metals in commercial fish from five fresh water lake Pakistan. Toxicology and Environmental Chemistry, 33: 133 - 140.

Tawari-Fufeyin P and Ekaye S, 2007. Fish species diversity as indicator of pollution in Ikpoba River, Benin City, Nigeria. Rev. Fish Biol. Fisher, 17: 21 - 30.

Tayel S, Yacoub A.M and Mahmoud S, 2008. Histopathological and haematological responses to freshwater pollution in the Nile Catfish *Clarias gariepinus*. J. Egypt Acad. Soc. Environ. Develop., 9: 43 - 60.

Terriere L.C, 1984. Induction of detoxication enzymes in insects. Annu. Rev. Entomol., 29: 71 - 88.

Torri S.I, Urricariet S and Lavado S.R, 2011. Micronutrient availability in crop soil of the pampas region, Argentina; In: Soil Nutrients; 978-1-61324 -785-3.

Uluturhan E and Kucuksezgin F, 2007. Heavy metal contaminations in Red Pandora (*Pagellus erythrinus*) tissues from the Eastern Aegean Sea, Turkey. Water Research, 41: 1185 - 1192.

Usero J, Gonzalez-Regalado E and Gracia I, 1996. Trace Metals in the Bivalve Mollusc Chamelea gallina from the Atlantic Coast of Southern Spain. Mar. Pollut .Bull., 32: 305 - 310.

Vallee B. L and Falchuk K. H, 1993. The biochemical basis of zinc physiology. Am Physiological Soc.

Vercoutere K, Fortunati U, Muntau H, Griepink B and Maier E.A, 1995. The certified reference materials CRM 142 R sewage sludge amended soil and CRM145 R sewage sludge for quality control in monitoring environmental and soil pollution. Fres. J. Anal. Chem., 352:197 - 202.

Wallace J.B and Webster J. R, 1996. The role of macroinvertebrates in stream ecosystem function. Anna Rev. Entomol., 41: 115 - 39.

WHO (2004): Guidelines for drinking - water quality, Geneva, Swizerland.

Wood J. M, 1974. Biological cycles for toxic elements in the environment. Science, 183, 10.

Woodbury P. B, 1998. Municipal Solid Waste Composting: Potential Effects of Heavy Metals in Municipal Solid Waste Composts on Plants and the Environment. Boyce Thompson Institute for Plant Research: Cornell University, Ithaca, NY.

Yayıntas O.T, Yılmaz S, Turkoglu M and Dilgin Y, 2007. Determination of heavy metal pollution with environmental physicochemical parameters in waste water of Kocab as Stream (Biga, Canakkale, Turkey) by ICP-AES. Environmental Monitoring and Assessment, 127: 389 - 397.

Zatta P, Ibn-Lkhayat-Idrissi M, Zambenedetti P, Kilyen M, and Kiss T, 2002. In vivo and in vitro effects of aluminum on the activity of mouse brain acetylcholinesterase. Brain Research Bulletin, 59: 41 - 45.

Zhou Q, Zhang J, Fu J, Shi J and Jiang G, 2008. Biomonitoring: An Appealing Tool for Assessment *Periophthalmus waltoni* Koumans 1941 (Gobiidae: of Metal Pollution in the Aquatic Ecosystem. Analytica Chimica Acta, 606 (2): 135 - 150.

Zyadah M.A, 1995. Environmental impact assessment of pollution in Lake Manzalah and its effect on fishes. Egypt, El-Mansoura University, 1 - 27.

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