Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS

Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2016.122.131

Research Article
Use of Oxidative Stress and Genotoxic Biomarkers of Aquatic Beetles *Anaceana globulus* (Coleoptera: Hydrophilidae) as Biomonitors of Water Pollution

[1]Mourad L. Shonouda, [1]Lamia M. El-Samad, [2]ELHassan Mokhamer and [2]Noura Toto

[1]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt
[2]Department of Zoology, Faculty of Science, Damanhour University, Damanhour, Egypt

Abstract
Background and Objective: Contamination of water lakes by toxic chemical pollutants is one of the largest threats to environment and human health. They occur in the environment as a result of natural processes and/or as pollutants from human activities. Lakes are important natural resources for agricultural crops and fish production particularly in the developing countries. In Egypt, lake Mariut is one of the aquatic ecosystems suffers extremely from almost all possible human activities, so it is considered the polluted site, while lake Edku is considered the reference one due to less impact of human activities and environmental problems. **Materials and Methods:** Insect samples of *Anaceana globulus* (Coleoptera: Hydrophilidae) were collected from reference and polluted lakes. Oxidative stressors and chromosomal aberrations of insect tissues were studied. Statistical analysis (student t-test) was applied to show the significance difference between data collected from both sites. **Results:** Water pollution of lake Mariut caused changing in water characteristics that lead to a great alteration in its physiochemical parameters. Heavy metals: Copper, zinc, manganese, iron, lead, cadmium and cobalt were also detected significantly in water samples and in insect body samples collected from the polluted site. The accumulated toxic heavy metals in body insect samples in addition to the unhealthy environmental physiochemical conditions lead to significant changes in oxidative stress biomarkers. These changes represented by a significant decrease in the activity of glutathione peroxidase, catalyase and glutathione content as well as the total protein content. On the other hand, significant increases in activities of aspartate transaminase, alanine transaminase and malondialdehyde concentration were recorded. Moreover, these pollutants induced significantly chromosomal aberrations such as fragments, stickiness, gaps and polyploidy. Additionally, binucleated and micronucleus cells were observed in the insect metaphase cells. **Conclusion:** The evaluation study of using aquatic beetles as a monitor of water pollution was reflecting the dramatically increasing of mutagenicity. Also, the damage by oxidative stress was probably pronounced related to the decreased antioxidant capacity. It gives a good indicator for the level of ecotoxicology and the severe impact of human activities on the selected habitats.

Key words: Biomarkers, chromosomal aberrations, environmental pollution, heavy metals, oxidative stress, water beetles

Received: February 18, 2016 Accepted: May 12, 2016 Published: June 15, 2016

Citation: Mourad L. Shonouda, Lamia M. El-Samad, ELHassan Mokhamer and Noura Toto, 2016. Use of oxidative stress and genotoxic biomarkers of aquatic beetles *Anaceana globulus* (Coleoptera: Hydrophilidae) as biomonitors of water pollution. J. Entomol., 13: 122-131.

Corresponding Author: Mourad L. Shonouda, Department of Zoology, Faculty of Science, Maharam Bey, Alexandria University, P.O. Box 21511, Alexandria, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The development of modern industry causes increasingly serious pollution in the surrounding environment. These pollutants may contaminate water, soil and air that released in the environment via human waste, industrial effluents, exhaust gases and/or agrochemicals. A dangerous situation may arise as some of these pollutants are mutagenic and causing a catastrophic health risk, including serious disease likes cancer[1].

The contamination of water resources by different chemical pollutants including heavy metals is a major concern due to their toxicity, persistence and bioaccumulation problems[2]. The most important heavy metals responsible of water pollution are Zn, Cu, Cd, Hg, Ni and Cr. Metal ions can be incorporated into food chains and concentrated in aquatic organisms to a level that affect their physiological state[3,4].

Aquatic environment represented by water lakes are more sensitive to pollution inputs because lakes flush out their contents relatively slowly. The Egypt's Northern delta includes different lakes situated on the Mediterranean coast of the delta and cover about 6% of the non-desert surface area of Egypt. Lakes are important natural resources for agricultural crops and fish production in Egypt[1].

One of these lakes is lake Edku lies Northeast of Alexandria. It receives mainly agricultural drain water and is considered one of the most important fish sources in Egypt[5]. The second important lake is lake Mariut located in the North of Egypt Southeast to Alexandria city and one of the most heavily populated urban areas in Egypt. Unfortunately, lake Mariut suffers from almost all possible environmental problems especially from human activities. Pollutants load has been came from sanitary drainage, industries and agricultural waste waters. According to these sources of pollution, lake Mariut is considered the most polluted one of Egypt[6].

Insects are one group of living organisms inhabiting these areas and have been used as bioindicators of pollution through assessment of sensitive biomarkers. Biomarkers represented by a changing in biological response ranging from molecular level to behavioral changes, which can be related to exposure to environmental contaminants[7]. Particularly, coleopteran insects constitute the most flourishing taxonomic order of the animal kingdom. Beetles were used in the present study for their highly adaptive characters for external environmental conditions, they maintain stable populations and are longed-lived[8,9] and able to survive under harsh environmental conditions as a result of physiological and behavioral adaptations[10]. Also, water beetles play a vital role in the aquatic environmental balance through its important role in the food chain. The genus *Anacaena* belongs to a large family of hydrophilic beetles, which is represented from all major biogeographically regions and the species seems to show no particular preference for water type. They may be more frequent at the margins of running water[11].

Organisms could respond to environmental stressors both behaviorally and by means of biochemical and physiological mechanisms[12,13]. The mechanisms by which metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of Reactive Oxygen Species (ROS)[14]. It is known that biochemical alterations resulted from the exposure to contaminants are early evidence for the negative effects of these contaminants[15]. Hence, the use of biomarkers in monitoring of environmental stressors represents a significant tool for evaluation of pollution in different organisms. The increase or decrease in the activity of certain enzymes can explain a possible response to the environmental stressors and can provide good qualitative evidences for damages caused by certain pollutants. Clearly, toxic compounds have high impact on the metabolic pathway mainly depending on enzymes reflecting the changes of enzyme activities[16]. Several studies have shown a great interest in the use of enzymatic biomarkers for monitoring of environmental stressors. Biomarkers that extensively used in the biochemical studies of environmental impacts are the superoxide dismutase (SOD), catalase (CAT), Glutathione Reductase (GR) and Glutathione-S-Transferase (GST)[17]. The first discovered enzyme among the enzymatic antioxidants was SOD that catalyzes the formation of hydrogen peroxide (H_2O_2) from superoxide ($O_2^{\bullet}$) and is generally, one of the first to act against damages caused by ROS[18]. In addition, the metabolism of reduced glutathione (GSH) is one of the main antioxidant defense mechanisms in the living systems[19]. Another important defense system against the increase of free radicals involves the glutathione peroxidase (GPx), which acts in the removal of H_2O_2 and lipid hydroperoxides (LOOHs) from the cell. One of the forms of GPx and the most studied detoxicant enzyme in different organisms is GST. It is considered as a detoxification enzyme because it metabolizes a great variety of xenobiotic compounds, catalyzing their conjugation with GSH and forming substances of low toxicity[20].

Chromosomal aberration is considered as one of the detecting genetic effects of environmental pollutants by examining mitotically active metaphase cells for numerical and structural changes of the chromosomes. The structural and numerical chromosomal aberrations were noticed in the coleopterous insects *Blaps sulcata* and *Akis reflexa* exposed to cement dust[21] and in the hemipterous insects *Sphaerodema urinator* exposed to heavy metals pollution[22]. Also, they found in the orthopteran insect *Tetrix tenuicornis*[23], in the dipteran *Prodiamesinae* species[24] and in the bow fly *Lucilia cuprina*[25].

According to these facts the present study aims to assay the water physicochemical characteristics and concentrations of different heavy metals accumulated in the body of aquatic beetles *Anacaena globulus* collected from lake Edku and lake Mariut. Also, evaluate their effect on oxidative stress and genotoxic markers in order to investigate the possibility of employing insects as sensitive indicators for aquatic environmental pollution.

MATERIALS AND METHODS

Insect sampling: Insects and water samples were collected from the littoral zone of lake Edku (reference site) and were also sampled from the littoral zone of lake Mariut, which is considered as a polluted site due to human impact. Five samples (each consisted of 20 U) of the water beetle, *Anacaena globulus* (Coleoptera: Hydrophilidae) were collected from the studied lakes. From each location, a total number of 200 individuals of the same size were sampled. Insects were collected in a metallic strainer (20 cm in diameter) with an aluminum handle before being transferred into glass containers filled with lake water according to the specific sampling locations. Insects were then transferred to the laboratory within an hour. Insects were washed in running water to remove any debris. Insects were kept at -20°C until experimental time. For each biological test, 20 individuals were polled.

Analysis of lake water: The water of each lake was analyzed to determine the chemical composition. Three random water samples were collected from each sampling location about 20 cm below water surface to avoid floating matter. Polyethylene bottles were used for sampling. Water samples were filtered in the field using a polypropylene syringe fitted with a 0.45 µm. Millipore cellulose acetate filter and acidified for preservation. Physical and chemical characteristics of the water samples including pH, Total Dissolved Salts (TDS), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), alkalinity, nutrient salts as phosphate (PO_4-P), ammonia (NH_4-N), nitrate (NO_3-N) and nitrite (NO_2-N) were measured by standard methods for the analysis of natural and treated waste water described by the American Public Health Association[26]. Concentrations of heavy metals: Copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), lead (Pb) and cobalt (Co) were measured in filtered lakes water according to Riley and Taylor[27] using graphite furnace atomic absorption spectroscopy (Berkin-Elmer model 2380) under the recommended conditions and the Detection Limits (DL) in the manual for each metal.

Preparation of insect body samples for biochemical analysis: Hundred milligrams of the whole insect was homogenized in 1 mL of cold potassium phosphate buffer solution (50 mM, pH 7.5 containing 1 mM of ethylene diamine tetra acetic acid (EDTA). Homogenized tissues were divided into two portions. One was used to assay malondialdehyde (MDA) concentration and total protein content. The other portion was centrifuged at 6000 rpm for 15 min at 4°C. The resulting supernatant was separated to assay the content of glutathione (GSH) and the activities of Glutathione Peroxidase (GPx) and catalase (CAT).

Oxidative stress biomarker

Malondialdehyde (MDA) concentration: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form TBA reactive product, the absorbance of the resultant pink product can be measured at 534 nm. Malondialdehyde (MDA) concentration was determined according to Ohkawa *et al.*[28].

AST, ALT enzymatic activity levels and total protein content: Aspartate tranaminase (AST) and alanine transaminase (ALT) activities in insect samples were measured colorimetrically according to method described by Tietz[29], while the total protein content was determined by applying the method described by Lowry *et al.*[30] using bovine serum albumin as a standard.

Antioxidant biomarkers

Reduced glutathione (GSH) assay: The method based on the reduction of 5, 5'dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH content and its absorbance can be measured[31] at 412 nm.

Glutathione Peroxidase (GPx) activity: The assay is an indirect measure of the activity of GPx. The GSSG produced upon the reduction of an organic hydroperoxide (ROOH) by GPx, which is recycled to its reduced state by the enzyme

Table 1: Physical and chemical characteristics of water collected from reference and polluted sites

Parameters	Reference site	Polluted site
pH	7.3600±0.029	7.150±0.34
TDS (mg L^{-1})	1358.4400±38.94	5282.040±36.20*
DO (mg L^{-1})	4.1800±0.33	2.360±0.25*
COD (mg L^{-1})	2.5000±0.32	172.260±2.29*
BOD (mg L^{-1})	1.8800±0.10	81.400±12.88*
Alkalinity (mg L^{-1})	201.2100±0.86	233.640±9.54*
PO_4-P (mg L^{-1})	0.0020±0.001	0.0080±0.003
NH_4-N (mg L^{-1})	0.0020±0.000	0.1650±0.017*
NO_3-N (mg L^{-1})	0.0430±0.005	0.0830±0.001*
NO_2-N (mg L^{-1})	0.0012±0.0023	0.0042±0.0061

*Statistically significant at ($p \leq 0.05$)

Table 2: Concentrations (Mean±SE) of heavy metals (mg L^{-1}) in water samples from either reference or polluted sites

Elements	Reference site	Polluted site
Cu	0.018±0.002	1.010±0.070*
Zn	0.067±0.013	0.543±0.057*
Cd	0.002±0.002	0.044±0.002*
Fe	0.142±0.012	1.010±0.070*
Mn	0.049±0.008	0.080±0.008*
Pb	0.145±0.008	1.010±0.031*
Co	0.021±0.004	0.067±0.006*

*Statistically significant at ($p<0.05$)

Glutathione Reductase (GR), alcohol (ROH) and water. The activity of GPx in the sample was determined according to Paglia and Valentine[32].

Catalase (CAT) activity: The enzyme catalase (CAT) converts H_2O_2 into water (H_2O). The CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 according to Aebi[33].

Chromosomal aberrations: Chromosomal aberrations were obtained from the analysis of metaphase in testes cells as they were difficult to get from ovarian cells. Adult males of the studied insect collected from each site (lake Edku and lake Mariut) were injected in the abdominal region with a dose of 0.05% colcemid for 3 h. Specimens were dissected in insect Ringer solution and testes were removed by fine forceps and immersed in 1.0% sodium citrate solution for 10 min at room temperature. After that, the testes were fixed in ethanol acetic acid (3:1) for at least 1 h.

Chromosomal analysis was carried out by squash technique used by Osman[21]. The dissected testes were stained with 2.0% aceto-orcein solution then fixed for about 10 min in 45.0% acetic acid. The testes were torn to pieces on a slide and the cover slip was squashed by applying pressure to it with the thump. Maximum pressure must be avoided so as not to move the cover slip. If the squash is not sufficient, acetic acid is added to the edge of the cover slip and squashing is repeated. The best-spread metaphase cells were selected. Chromosome number and structure were examined in 100 intact metaphase cells of the studied groups. The type and frequency of numerical (polyploidy and aneuploidy) as well as structural (gap, stickiness, fragment, break, deletion, ring, ect.) aberrations in the studied insect collected from each site were recorded.

Statistical analysis: The difference between mean values of each mentioned parameter of the two studied sites was statistically tested for significance at $p<0.05$ using the student's t-test[34].

RESULTS

Physicochemical characteristics of water samples: Table 1 showed the physical and chemical characteristics of the water collected from the polluted lake Mariut and from the reference lake Edku. Values of pH were more-or-less neutral but slightly on the alkaline side in the two sites. Water samples collected from lake Mariut revealed significant ($p<0.05$) higher concentrations of Total Dissolved Salts (TDS) (5282.04±36.20), Chemical Oxygen Demand (COD) (172.26±2.29), Biochemical Oxygen Demand (BOD) (81.40±12.88) and alkalinity (233.64±9.54) as compared to those of lake Edku. Moreover, the obtained data of chemical analysis from polluted lake Mariut revealed a significant increase ($p<0.05$) in the concentrations of nutrient salts: Nitrate (0.165±0.017) and ammonia (0.083±0.001), while nitrite is higher (0.0042±0.0061) but not significant as compared to those of lake Edku. On the other hand, Dissolved Oxygen (DO) (2.36±0.25) is significantly lower ($p<0.05$) than that of polluted reference site (4.18±0.33).

Concentration of heavy metals in water samples: Table 2 revealed that the mean concentrations of copper (Cu) (1.010±0.070), zinc (Zn) (0.543±0.057), iron (Fe) (1.010±0.070), manganese (Mn) (0.080±0.008), lead (Pb) (1.010±0.031), cobalt (Co) (0.067±0.006) and cadmium (Cd) (0.044±0.002) were significantly ($p<0.05$) higher in water samples collected from lake Mariut (polluted) when compared with those of lake Edku (reference).

Concentrations of heavy metals in insect body: The present results revealed that there was accumulation of heavy metals in the body of insects. The mean concentrations of the heavy metals, Cu (0.0223±0.0004), Zn (0.067±0.013), Fe (0.543±0.057), Mn (0.0629±0.0064), Pb (0.0581±0.0183), Co (0.0014±0.0002) and Cd (0.016±0.005) were significantly ($p<0.05$) higher in insect samples collected from lake Mariut (polluted site) as compared to insect samples collected from lake Edku (reference site) as shown in Table 3.

Biochemical analysis

Malondialdehyde (MDA) concentration: The results demonstrated variation in the biochemical parameters in tissues of the studied insects in response to pollution. The results concerning MDA concentration in insect body samples of the two studied sites clearly showed that there was a significant increase ($p<0.05$) in MDA concentration in insect body collected from the polluted site (9.70±1.14) when compared with that of the reference one (3.56±0.30) (Table 4).

Aspartate transaminase (AST) and alanine transaminase (ALT) activities: The results of AST and ALT activities in insect body samples of studied sites confirmed that there was a highly significant increase ($p<0.05$) in the both enzyme activities in insect body collected from polluted site (199.60±10.59, 127.20±12.04) when compared with that of the reference one (121.80±10.16, 77.20±4.85), respectively, as shown in Table 4.

Table 3: Concentrations (Mean±SE) of heavy metals (µg g^{-1} dry weigh) in insect body samples from either reference or polluted sites

Elements	Reference site	Polluted site
Cu	0.0093±0.0014	0.0223±0.0004*
Zn	0.0020±0.002	0.0670±0.013*
Cd	0.0008±0.0003	0.0160±0.005*
Fe	0.0010±0.001	0.5430±0.057*
Mn	0.0211±0.0044	0.0629±0.0064*
Pb	0.0008±0.0001	0.0581±0.0183*
Co	ND	0.0014±0.0002*

*Statistically significant at ($p<0.05$), ND: Not detected

Table 4: Concentrations (Mean±SE) of MDA, GSH and TP in addition to enzymatic activities (Mean±SE) of AST, ALT, GPx and CAT in insect body samples collected from reference and polluted sites

Parameters	Reference site	Polluted site
MDA (nmol mg^{-1} tissue)	3.56±0.30	9.70±1.14*
AST (U mg^{-1} protein)	121.80±10.16	199.60±10.59*
ALT(U mg^{-1} protein)	77.20±4.85	127.20±12.04*
TP content (mg g^{-1} tissue)	152.60±11.09	107.00±6.36*
GSH (mU mg^{-1} protein)	30.80±2.46	21.00±2.35*
CAT (U mg^{-1} protein)	61.80±3.71	37.00±4.16*
GPx (mU mg^{-1} protein)	49.60±3.96	32.20±3.71*

*Statistically significant at ($p<0.05$), U: The enzyme quantity that decompose 1 µmol of substrate in 1 min at optimum conditions of the reaction, TP: Total protein

Total protein content: Table 4 represented the results concerning total protein content in insect body samples collected from the studied sites. The statistical analysis demonstrated that there was a high significant decrease ($p<0.05$) in total protein content in insect body collected from the polluted site (107.0±6.36) than that of the reference one (152.60±11.09).

Antioxidant biomarkers: The results demonstrated variation in different antioxidant biomarkers in tissues of the studied insects in response to pollution. The results showed reduction in the level of glutathione (GSH) content (21.0±2.35), catalase (CAT) activity (37.0±4.16) and glutathione peroxide (GPx) activity (32.20±3.71) in insect body samples from polluted studied site. The difference between means of the three antioxidant biomarkers was highly significant ($p<0.05$) in samples collected from the polluted site in compare to the reference one (30.80±2.46, 61.80±3.71 and 49.60±3.96, respectively) (Table 4).

Chromosomal aberrations: Chromosome numbers of *Anaceana globulus* were 2n = 14+xx, xy[35]. Cytological examinations in testes cells of the studied insect revealed that types of chromosomal aberrations are structural and numerical. The structural aberrations are fragments (0% reference, 6% polluted), stickiness (4% reference, 40% polluted) and gaps (2% reference, 8% polluted) while the numerical aberrations are polyploidy (1% reference, 10% polluted). Also, micronucleus and binucleated cells were observed in insect cells sampled from the polluted sites. Table 5 summarized the percentages of several types of aberrations as well as the percentage of total aberrant metaphases in testes cells of insects collected from the polluted sites. It is clear from the table that all types of aberrations were significantly increased in testes cells of insect collected from polluted site in compare to reference one. The percentages of total aberrant metaphases ranged

Table 5: Percentage of chromosomal aberrations of testes metaphase cells collected from reference and polluted sites

	Types of chromosomal aberrations (%)					
Sites	Stickiness	Fragments	Polyploidy	Gap	Total aberrations (%)	χ^2
Reference	4	0	1	2	7	45.8
Polluted	40	6	10	8	64*	

*Significance at $p<0.01$

from 7% (reference site) to 64% (polluted site). Chi-square (χ^2) test showed that the differences in percentage total aberrant metaphase between the two sites were highly significant ($p<0.01$).

DISCUSSION

There were insignificant difference values of pH, nutrient salts phosphate (PO_4-P) and nitrite (NO_2-N) between both sites meaning these parameters had no adverse effect on the metabolism of selected insects while, there were significant differences between the other parameters. The present level of Total Dissolved Salts (TDS) in polluted site was significantly higher than that of reference site. The increase in TDS in polluted site may be a result of mixed salty effluents of industrial, domestic and agricultural wastes. This significance increase in TDS levels affects different biological processes such as growth, reproduction and survival of aquatic organisms[36].

The significant decrease of Dissolved Oxygen (DO) level in water samples of lake Mariut could be as a result of intensive load of organic pollutants[37] and the decrease in environmental oxygen concentrations resulting from the acute effect of water pollution leading to induce oxidative stress[4]. Also, the exposure to high concentrations of organic and inorganic pollutants leads to increase the Chemical Oxygen Demand (COD) and the Biochemical Oxygen Demand (BOD) that use to measure the quality of water. The increase significantly of COD and BOD gives a clear indication for highly polluted area as shown in lake Mariut[26]. Additionally, one of physiochemical parameters that indicate the pollution of aquatic environmental is the alkalinity. Alkalinity was observed to be increased significantly in lake Mariut and it was known that water far away from pollution had low alkalinity[37].

Another parameter is nutrient salts that introduced into aquatic environments with sewage effluents. The higher level of ammonia had hazardous effect on the aquatic life as shown in polluted lake Mariut while, lake Edku had trace amount of ammonia may be due to the shallowness of the lake and well aeration of water, which causes oxidation of ammonia to other higher oxidized forms of nitrogen[38]. These clearly changes in water characteristics due to the toxic effluents from industrial and agricultural activities accompanied by increasing the concentrations of heavy metals.

The present results showed accumulation of lower concentrations of metals copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), lead (Pb), cadmium (Cd) and cobalt (Co) in the body of adult beetle *Anacaena globulus* sampled from the studied sites, relative to their concentrations in water. No records of cobalt (Co) accumulation were found in body of adult insects as found in water samples of the reference site. It is clear that beetles have a great resistance to environmental pollution and have the ability to eliminate the toxic pollutants through several ways, however, in the present study, the accumulated traces of toxic heavy metals in the insect body samples showed significant changes in the metabolism of insect body indicated by the changes in the oxidative defense system of the mentioned aquatic beetle.

Accumulations of different toxic heavy metals (Cu, Zn, Fe, Mn, Pb, Cd and Co) in the body beetle *A. globulus* collected from the polluted site induce detoxification processes to lower these pollutant levels. Insects possess a suite of antioxidant enzyme systems like other animals which protect their cells from the damaging effects of oxidative radicals. Oxidative stress in insects may result from an imbalance of oxidants and antioxidants under a significant impact of metals. The mechanisms by which metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of Reactive Oxygen Species (ROS)[14]. For example, the toxic action of cadmium causes oxidative damage to DNA, proteins and lipids[39].

The heavy metals pollution increased clearly malondialdehyde (MDA) concentration of the studied insects. The increased concentration of MDA may be due to the highly ROS, especially HO which acts on polyunsaturated fatty acids (PUFA) of membranes to produce MDA[40]. Also, the increased formation of MDA concentration is mediated by both the increase in the induced ROS and the inhibition of superoxide dismutase (SOD) and CAT activities[41,42]. Heavy metal cadmium pollution certainly increases significantly MDA level in many insects such as *Oxya chinensis* (Orthoptera: Acridoidea)[43] and *Blaps polyresta* (Coleoptera: Tenebrionidae)[13]. Also, there was a strong positive correlation between different concentrations of Pb in dragon fly *Austroaeschna inermis* and all enzymes and MDA concentration[44], suggesting that increases in its concentration may be linked with increased cell membrane damage in insect and subsequently oxidative stress. Moreover, the heavy metal Zn leads to dramatic changes in the antioxidant enzymes to reduce detoxification mechanisms which cause the mortality of hemipteran insect *Odontopus varicornis*[16].

Concerning enzymatic activity, the present results pointed out a significant increase in AST and ALT activities in tissues of the polluted insects. It is well known that tissue damaged by toxicants exhibits a sharp rise in AST and ALT activities. After exposure to metal pollution the proteins degraded by proteolysis due to increase of the protease activity and the suppression of protein synthetic capabilities can elevate the free amino acid concentration[45].

Regarding the total protein content, there was a remarkable decrease in insect body samples of the polluted site. Proteins are not only biological compounds that regulate and integrate several physiological and metabolic processes in the body but also play a major role in the synthesis of detoxifying enzymes and help to detoxify the entered toxicants[46]. Consequently, the decrease in total protein content may reflect the decrease in the activities of some enzymes. This fact confirms also the results of GSH, GPx and CAT. Moreover, the decrease in protein content could be due to the breakdown of protein into amino acids and with the entrance of these amino acids to Krebs cycle as keto acid to supply energy for the insect[13]. Concerning the antioxidant biomarkers, the present results indicated a significant decrease in GSH content in tissues of the studied insects in response to pollution. The reduction in GSH content may be indicated by the high level of lipid peroxidation (LPO) due to the overproduction of ROS[47,48]. Furthermore, the reduction of GSH content may be due to their consumption in the scavenging free radicals probably generated by heavy metals[49,13].

Water pollution exhibited a remarkable significant reduction in CAT activity in the insect body samples of the polluted site. The present finding agreed with Augustyniak and Migula[50] who concluded that Cd reduced the activity of CAT in the grasshopper *Chorthippus brunneus*. The decline in the CAT activity may be due to reducing the conversion of $O_2^{\bullet}$ to H_2O_2 by SOD, which then leads to the accumulation of $O_2^{\bullet}$. Thus there is a certain relationship between CAT and SOD because SOD can convert the free $O_2^{\bullet}$ to H_2O_2, which is then eliminated by CAT. Also, it was found water pollution by different toxic heavy metals decrease GPx activity in the insect body samples of the polluted site. Decreasing the activity of GPx indicates its reduction capacity to scavenge H_2O_2 and LOOHs[51].

Generally, the decrease of the total protein may reflect the decrease in the activities of some enzymes in this study such as SOD, GPx and CAT. It was found that the activity of GSH-dependent enzymes decreased in insects that exposed to metals because GSH binds heavy metals, thereby protecting molecules such as DNA, RNA and proteins[50]. When Cd accumulates in the insect, it can combine with the active center (Se-Cys) of GPx and lead to structural changes that inactivate GPx[52]. The results of biomarkers for antioxidative defense, such as Glutathione Peroxide (GPx) activity, glutathione (GSH) content, malondialdehyde (MDA) concentration and total soluble protein contents revealed that metabolism is largely directed towards defense against ROS. The significant changes in all mentioned parameters, together with total soluble protein contents confirm that the damage by oxidative stress was probably pronounced related to the decreased antioxidant capacity. This is due to the effect of heavy metals water pollution in addition to the unhealthy environmental conditions represented by changing in physicochemical characteristics specifically in the polluted site.

The analysis of chromosomal aberrations proved that heavy metal pollutants capable to induce significant increase in abnormal metaphase cells of the studied insect. The studied insects were found to contain high frequency of stickiness, fragments, polyploidy and gap. Such results give evidence that heavy metals have a severe clastogenic effects upon the insect genome. Chromosomal aberrations in the present study may be appeared as a result of interaction of heavy metal toxicants with DNA and other cellular macromolecules[50]. Toxic chemicals can react with nucleotide base in DNA leading to DNA lesions which responsible for the production of chromosomal aberrations. Chromatid gaps and breaks can occur as a result of disrupt of animal cells life cycle[53]. Also, the cytogenetic technique commonly used for the evaluation of genotoxicity effects caused by chemical stressors. Binucleated and micronuclei are formed during cell division when complete or fragment chromosomes fail to be incorporated in the daughter nuclei, forming small additional nuclei.

It is important at the end to point out that heavy metals are capable to induce DNA damage as a consequence of dramatic chromosomal aberrations in germinal cells of the insect. Such results can reflect the dramatically increasing of mutagenicity, subsequently; the risk of this mutagenicity is changing in the genetic material that could not only have a magnified effect but also possibly an everlasting one.

CONCLUSION

The aquatic ecosystems are unique because of their important ecological characteristics. Therefore, any remaining ecosystem should be placed high on the list of conservation priorities and environmental safety. For that, we recommended to use living insects especially water beetles as a good model for bio-warning monitoring water pollution. Different bioindicator parameters provide an effective measure for early environmental stresses due to their sensitivities for even low levels of pollution.

SIGNIFICANCE STATEMENTS

Environmental pollution is a serious problem facing many countries especially the developing ones. Toxic chemical pollutants lead to hazardous effect for the general health of human beings. Aquatic environment represented by lakes are more sensitive to chemical pollutants at the same time they are important natural sources of fish production particularly in Egypt. Heavy metals are a group of pollutants accumulate in the body of different living organisms. The present findings showed that even the traces of different heavy metals change the physiochemical characters of water and affect dramatically the metabolism of organisms. These changes indicated by significant alterations of oxidative stress and genotoxic parameters. Using living organisms represented by insect water beetles is a good bio-warning monitor for water pollution.

REFERENCES

1. Saeed, S.M. and I.M. Shaker, 2008. Assessment of heavy metals pollution in water and sediments and their effect on *Oreochromis niloticus* in the Northern Delta Lakes, Egypt. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-14, 2008, Cairo, Egypt, pp: 475-490.
2. Franca, S., C. Vinagre, I. Cacador and H.N. Cabral, 2005. Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the tagus estuary (Portugal). Mar. Poll. Bull., 50: 998-1003.
3. Saad, M.A.H., 2003. Impact of diffuse pollution on the socio-economic development opportunities in the Costal Nile Delta lakes. Proceedings of the 7th IWA International Specialised Conference on Diffuse Pollution and Basin Management, August 17-21, 2003, Dublin, Ireland, pp: 81-85.
4. Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol., 101: 13-30.
5. Mohamed, E.A., 2003. Study of some chemical and physical changes of water and sediments of Lake Edku. M.Sc. Thesis, Alexandria University, Egypt.
6. Mateo, M.A., 2009. Lake mariut: An ecological assessment. Centro de Estudios Avanzados de Blanes, Consejo Superior de Investigaciones Cientificas, Blanes, Spain.
7. Abdel-Gawad, F.K., M.A. El-Seehy and M.M. El-Seehy, 2010. Genotoxic effect, biomarkers and aquatic contaminants in *Tilapia*. World J. Fish Mar. Sci., 2: 327-334.
8. Wise, D.H., 1981. Seasonal and yearly patterns in the densities of darkling beetles (Coleoptera: Tenebrionidae) in a montane community. Environ. Entomol., 10: 350-358.
9. Osman, W., L.M. El-Samad, E.H. Mokhamer, A. El-Touhamy and M. Shonouda, 2015. Ecological, morphological and histological studies on *Blaps polycresta* (Coleoptera: Tenebrionidae) as biomonitors of cadmium soil pollution. Environ. Sci. Pollut. Res., 22: 14104-14115.
10. Carrara, R., D.P. Vazquez and G.E. Flores, 2011. Habitat specificity can blur the predictions of species-energy theory: A case study of tenebrionid beetles adapted to aridity. J. Arid Environ., 75: 703-710.
11. Hansen, M., 1999. Hydrophiloidea (Coleoptera). In: World Catalogue of Insects, Hansen, M. (Ed.)., Vol. 2, Apollo Books, Copenhagen.
12. Migula, P., P. Laszczyca, M. Augustyniak, G. Wilczek, K. Rozpedek, A. Kafel and M. Woloszyn, 2004. Antioxidative defence enzymes in beetles from a metal pollution gradient. Biologia, 59: 645-654.
13. El-Samad, L.M., M. El-Hassan, W. Osman, A. Ali and M.L. Shonouda, 2015. The ground beetle, *Blaps polycresta* (Coleoptera: Tenebrionidae) as bioindicator of heavy metals soil pollution. J. Adv. Biol., 7: 1153-1160.
14. Viarengo, A., 1989. Heavy metals in marine invertebrates: Mechanism of regulation and toxicity at the cellular levels. Rev. Aqua. Sci., 1: 295-317.
15. Fontanetti, C.F., L.R. Nogarol, R.B. de Souza, D.G. Perez and G.T. Maziviero, 2011. Bioindicators and Biomarkers in the Assessment of Soil Toxicity. In: Soil Contamination, Pascucci, S. (Ed.). InTech, Rijeka, Croatia, ISBN: 9789533076478, pp: 143-168.
16. Kumar, T.R., D.M. Emerald and S. Sethuraman, 2014. Impact of heavy metal zinc on enzyme studies in selected tissues of *Odontopus varicornis* (Dist.) (Hemiptera: Pyrrhocoridae). Int. J. Recent Scient. Res., 5: 1717-1728.
17. Mishra, S., S. Srivastava, R.D. Tripathi, R. Kumar, C.S. Seth and D.K. Gupta, 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere, 65: 1027-1039.
18. Nordberg, J. and E.S.J. Arner, 2001. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Radic. Biol. Med., 31: 1287-1312.
19. Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 160: 1-40.
20. Malmezat, T., D. Breuille, P. Capitan, P.P. Mirand and C. Obled, 2000. Glutathione turnover is increased during the acute phase of sepsis in rats. J. Nutr., 130: 1239-1246.
21. Osman, W.E., 2002. Studies of ecological variation on the genetic structure and some aspects of population characteristics of the beetles *Blaps sulcata* and *Axis reflexa* (Coleoptra: Tenebrionidae) in the Mediterranean coastal desert of Egypt. Ph.D. Thesis, Faculty of Science, Alex University, Egypt.

22. Kheirellah, D.A., 2006. Impact of pollution on the water bug *Sphaerodema urinator* (Dufour, 1833) inhabiting lakes Mariut and Edku. Ph.D. Thesis, Faculty of Science, Alexandria University, Egypt.
23. Warchalowska-Sliwa, E., M. Niklinska, A. Gorlich, P. Michailova and E. Pyza, 2005. Heavy metal accumulation, heat shock protein expression and cytogenetic changes in *Tetrix tenuicornis* (L.) (Tetrigidae, Orthoptera) from polluted areas. Environ. Pollut., 133: 373-381.
24. Michailova, P.V., J. Ilkova and K. White, 2003. Cytogenetic alterations in *Prodiamesinae* species (Diptera, Chironomidae) from different polluted regions. Folia Biologica, 51: 69-79.
25. Joshi, A. and P.K. Tiwari, 2000. Chromosomal responses of blowfly *Lucilia cuprina* to heat and heavy metal stress. Genetica, 109: 211-218.
26. APHA., 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edn., American Public Health Association, Washington, DC., USA.
27. Riley, J.P. and D. Taylor, 1968. Chelating resins for the concentration of trace elements from sea water and their analytical use in conjunction with atomic absorption spectrophotometry. Analytica Chimica Acta, 40: 479-485.
28. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
29. Tietz, N., 1976. Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadephia.
30. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
31. Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.
32. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.
33. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
34. Puri, B., 2002. SPSS in Practice: An Illustrated Guide. 2nd Edn., CRC Press, Boca Raton, FL., ISBN: 978-0340761120, Pages: 184.
35. Shaarawi, A.F. and B.R. Angus, 1990. A chromosomal investigation of five European species of *Anacaena thomson* (Coleoptera: Hydrophilidae). Insect Syst. Evol., 21: 415-426.
36. Anderson, J., T. Estabrooks and J. McDonnell, 2000. Duluth metropolitan area streams snowmelt runoff study. Stream Water Quality Assessment Technical Report, Minnesota Pollution Control Agency, March 2000, USA.
37. El-Rayis, O.A., M.A. El-Sabrouti and H.M. Hanafi, 1994. Some hydro-chemical observation from Lake Mariut prior to diversion of sewage of Eastern districts of Alexandria. Proceedings of the 1st Arab Conference on Marine Environment Protection, (MEP'94), Alexandria, pp: 205-219.
38. Masoud, M.S., A.A. Elewa, A.E. Ali and E.A. Mohamed, 2004. Metal distribution in water and sediments of Lake Edku, Egypt. Egypt. Sci. Mag., 1: 13-22.
39. Beyersmann, D., 2002. Effects of carcinogenic metals on gene expression. Toxicol. Lett., 127: 63-68.
40. Mansour, S.A. and A.T.H. Mossa, 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pestic. Biochem. Physiol., 93: 34-39.
41. Gultekin, F., M. Ozturk and M. Akdogan, 2000. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). Arch. Toxicol., 74: 533-538.
42. Aslanturk, A., S. Kalender, M. Uzunhisarcikli and Y. Kalender, 2011. Effects of methidathion on antioxidant enzyme activities and malondialdehyde level in midgut tissues of *Lymantria dispar* (Lepidoptera) larvae. J. Entomol. Res. Soc., 13: 27-38.
43. Zhang, Y., G. Sun, M. Yang, H. Wu and J. Zhang *et al*., 2011. Chronic accumulation of cadmium and its effects on antioxidant enzymes and malondialdehyde in *Oxya chinensis* (Orthoptera: Acridoidea). Ecotoxicol. Environ. Saf., 74: 1355-1362.
44. Ihechiluru, N.B., A.N. Henry and I.E. Taiwo, 2015. Heavy metals bioaccumulation and oxidative stress in *Austroaeschna inermis* (dragon fly) of the Lagos urban ecosystem. J. Environ. Chem. Ecotoxicol., 7: 11-19.
45. Li, B., Y. Xie, Z. Cheng, J. Cheng and R. Hu *et al*., 2012. Cerium chloride improves protein and carbohydrate metabolism of fifth-instar larvae of *Bombyx mori* under phoxim toxicity. Biol. Trace Elem. Res., 150: 214-220.
46. Wikinson, C.F., 1976. Insecticide Biochemistry and Physiology. Plenum Press, New York, USA., Pages: 431.
47. Arthur, J.R., 2000. The glutathione peroxidases. Cell. Mol. Life Sci., 57: 1825-1835.
48. Hossain, S.U. and S. Bhattacharya, 2006. Prevention of cadmium induced lipid peroxidation, depletion of some antioxidative enzymes and glutathione by a series of novel organoselenocyanates. Environ. Toxicol. Pharmacol., 22: 298-308.
49. Mircic, D., D. Blagojevic, V. Peric-Mataruga, L. Ilijin, M. Mrdakovic, M. Vlahovic and J. Lazarevic, 2013. Cadmium effects on the fitness-related traits and antioxidative defense of *Lymantria dispar* L. larvae. Environ. Sci. Pollut. Res., 20: 209-218.

50. Augustyniak, M. and P. Migula, 2000. Body burden with metals and detoxifying abilities of the grasshopper-*Chorthippus brunneus* (Thunberg) from industrially polluted areas. Trace. Metals Environ., 4: 423-454.
51. Wu, H., J. Liu, R. Zhang, J. Zhang, Y. Guo and E. Ma, 2011. Biochemical effects of acute phoxim administration on antioxidant system and acetylcholinesterase in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). Pest. Biochem. Physiol., 100: 23-26.
52. Yan, B., L. Wang, Y.Q. Li, N. Liu and Q. Wang, 2007. Effects of cadmium on hepatopancreatic antioxidant enzyme activity in freshwater crab *Sinopotamon yangtsekiense*. Acta Zoologica Sinica, 53: 1121-1128.
53. Brusick, D., 1980. Principles of Genetic Toxicology. Plenum Press, New York, USA.