ISSN 2347-6893

The ground beetle, Blaps polycresta (Coleoptera:Tenebrionidae) as Bioindicator of Heavy Metals Soil Pollution.

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ABSTRACT

Human activities can have dramatic impacts on animal populations around urban areas with heavy metal contamination being a primary cause of hazardous effects. Insects as residents of ecosystems are especially susceptible to heavy metal contamination and have the potential to serve as indicators for environmental stresses .To better understand the effect of heavy metals pollution on terrestrial insect, the detection of different heavy metals was investigated. The following metals cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb) were found and their concentrations were estimated in soil samples from either polluted or reference site. As the Cd concentration was significantly high in the polluted site, its concentration in the tissues of the studied insect *Blaps polycresta* (Coleoptera:Tenebrionidae), was investigated as well as the antioxidant defense system and lipid peroxidation biomarkers. The results of insect's tissues in polluted site showed a significant decrease in the activity of antioxidant enzymes, glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and reduction in the level of glutathione (GSH). In addition, there was a significant decrease in the total protein content. On the other hand a significant increase of transaminases (AST, ALT), and lipid peroxidation (LPO) levels was found. In conclusion, insect can be considered as a good bioindicator species for environmental heavy metals pollution especially by cadmium that accumulates in soft tissues and has deleterious effects.

Indexing terms/Keywords

Heavy metals; soil pollution; bioaccumulation; biomarkers; oxidative stress; *Blaps polycresta*; cadmium; Antioxidant enzymes.

Academic Discipline And Sub-Disciplines

Environmental biology; biochemical biology,;soil biology.;Zoology; toxicology and tolerability.

SUBJECT CLASSIFICATION

Enviromental biology

TYPE (METHOD/APPROACH)

Monitoring

Council for Innovative Research

Peer Review Research Publishing System

Journal: JOURNAL OF ADVANCES IN BIOLOGY

Vol 4, No.3

editor@cirjab.org

www.cirjab.com , editorsjab@gmail.com

ISSN 2347-6893

1. INTRODUCTION

Heavy metals are an important category of pollutants and as such have major detrimental impacts on both human health [1] and the health of terrestrial and aquatic communities and ecosystems. [2] Heavy metal pollution is ubiquitous in our environment and results from diverse activities such as phosphate fertilizers, industrial effluents, foundry wastes, paints, auto-mobiles, mining and rock weathering.

The exposure to these pollutants can intensify the production of reactive oxygen species (ROS), which are normally produced in non-stressed cells. However, their excess can lead to oxidative stresses by interacting with and damaging the structure of the DNA molecule causing harmful effects.[3]

Cadmium (Cd) is a persistent environmental pro-oxidant, which produces a wide variety of detrimental effects in organisms. Being non- degradable, it is accumulated in tissues of organisms, providing the disturbance of their physiological status. [4]

Insects constitute an important group of organisms, because of the diversity of their morphology and physiology, their abundance, economic importance and numerous effects on human health.[5] For that reason, insects have the potential to serve as indicators for environmental stresses.

There are examples of herbivorous insects, which are able to accumulate high amounts of Cd like Locusta migratoria, Oxya chinensis, Acrida chinensis (Orthoptera), Eligma narcissus or Lymatria dispar (Lepidoptera) larvae. [6-8]

Recent studies have shown a great interest in the use of enzymatic biomarkers for monitoring of environmental stresses. Biomarkers that extensively used in the biochemical studies of environmental impacts are the superoxide dismutase, catalase, gluthathione reductase and gluthathione-S-transferase. In addition, the metabolism of glutathione is one of the main antioxidant defense mechanisms in the living systems.[9] Another important defense system against the increase of free radicals involves the enzyme gluthathione peroxidase, which acts in the removal of hydrogen peroxide and lipid peroxides from the cell. [10]

For a long time, the insect fauna of Egypt have attracted the attention of many entomologists. Coleoptera constitutes the most flourishing taxonomic order of the animal kingdom. Tenebrionidae is one of the largest coleopterous families. It has a worldwide distribution. Many tenebrionids have succeeded in colonizing dry habitats. They are highly variable in shape and size, and are frequently dark. [11] They have economic significance by damaging young plants. Our study has been focused to investigate the possibility of employing insects as sensitive indicators or monitors for environmental stresses by the determination of biochemical parameters of tenebrionid beetles disturbed by environmental stress of pollutants.

2. MATERIALS AND METHODS

2.1. Insect

Insects were randomly distributed into two groups. Group I; comprised 20 adult insects (males and females) collected from the reference site (garden of the faculty of science at Moharrem Bey area, Alexandria). Group 2; included 20 adult insects (males and females) collected from Abis area which is situated in the vicinity of the town of Alexandria that represent the polluted site.

2.2. Chemicals

All chemicals were of analytical grade and kits for estimation of superoxide dismutase (SOD), catalase (CAT), glutathione-S- transferase (GST), aspartate transaminase (AST) and alanine transaminase (ALT) activities as well as kits for estimation of total protein content, glutathione content (GSH) and Malondialdehyde (MDA) concentration level were purchased from Biodiagnostic (29,tahrir St., Dokki, Giza,Egypt).

2.3. Analytical Methods

2.3.1. Determination of heavy metal concentrations in soil

Soil samples were collected from each site at a depth of 0-30 cm. Preliminary studies pointed out that copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd) are the most dominant heavy metals in the two selected sites. The concentration levels (µg/g) of these four metals were determined in five replicates of soil samples from the two sites. 5.0 g of the soil samples was decomposed in Teflon bomb using the total decomposition procedure. [12] Metal concentration was then estimated by using atomic absorption Spectrophotometer (Berkin-Elmer, 2380) in the faculty of science central laboratory, Alexandria University.

2.3.2. Determination of cadmium concentration in insect tissues

Alimentary canal and gonads (testis and ovary) were selected as target insect tissues to determine the concentration of cadmium (heavy metal with highest concentration in soil). The same mentioned method of metal analysis in soil was conducted for the selected tissues using 0.5 g of finely ground dry insect sample. Ten replicates were applied for each organ.

2.3.3. Biochemical analysis of tissue homogenate

2.3.3.1. Preparation of tissue homogenate.

Prior to dissection, tissues were perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml. heparin, and then the specimens were dissected to isolate tissue organs (ovary, testis, male and female alimentary canal). The tissue organs were homogenized in 5 ml of cold buffer (50 mM. of potassium phosphate, pH 7.5, containing 1mM.EDTA) per gram tissue. Then the tissues homogenate were Centrifuged at 10,000 × g for 15 minutes at 4oc and the resultant supernatant was used for the determination of different enzyme assays and thiobarbituric acid reactive substances (TBARS).

2.3.3.2. Lipid peroxidation (LPO) and reduced glutathione(GSH) assays.

According to the method of Ohkawa et al., the extent of lipid peroxidation in terms of TBARS formation was measured.[13] As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is 1.56 × 105M−1 cm−1. While GSH content was quantified according to the method of Beutler [14]

2.3.3.3. Determination of glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) enzymatic activity levels.

Glutathione S-transferase (GST; EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig et al.[15] Superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to the procedure of Nishikimi et al.[16] The assay procedure involves the inhibition of phenazine methosulphate (PMT)-mediated reaction of nitroblue tetrazolium (NBT) dye. One unit of enzyme activity is defined as the amount of enzyme that causes half-maximal inhibition of NBT reduction. The enzyme catalase (CAT; EC 1.11.1.6) converts H2O2 into water. The CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H2O2 according to Aebi.[17]

2.3.3.4. Determination of AST& ALT enzymatic activity levels and total protein content.

ALT and AST activities in tissues were measured colorimetrically according to method described by Reitman et al.[18] The protein content was determined by following the method described by Lowry et al.[19] using bovine serum albumin as a standard.

3. STATISTICAL ANALYSIS

Data were analyzed using SPSS 11.0 for windows. Significance was calculated using Mann Whitney U test. $P < 0.05$ was considered statistically significant.

4. RESULTS

4.1. Heavy metals concentrations in soil

Heavy metal concentrations at the reference site were fairly allied with the established standard levels except for cadmium. On the other hand, soil at polluted site exhibited higher concentration levels of Cd and Pb than the established standard levels. Soil samples from the polluted site were higher than the reference one in Cd, Pd and Zn being significantly different for Cd concentration only. For the other heavy metals concentrations, the difference between the two sites was insignificant (Table.1).

Table 1. Heavy metals concentrations (µg/g dry weight) in soil samples collected from the selected sites.

	Cd	Cu	Pb	Zn
Reference site	4.80	23.60	32.80	57.40
Polluted site	59.00*	20.80	40.00	63.00
Background[(a)]	0.62	25.80	29.20	59.80

Values are expressed as means ± standard errors.

(*) : Differ significantly as compared with reference ($P<0.05$).

(a) : Background values (Ure and Berrow 1982).[20]

ISSN 2347-6893

4.2. Heavy metals concentrations in insects

As cadmium was the only heavy metal that showed a significant increase in concentrations in soils of the polluted sites relative to other metals, it was helpful to determine its concentrations in gonads (testis and ovary) and alimentary canal of both sexes of the studied insects at the two selected sites.

Table 2 demonstrated that cadmium concentrations in gonads and alimentary canal of both sexes of the insects were lower than that of the soil. However, insects from polluted site contained higher amount of cadmium in comparison to the reference one, which may reflect the concentration of cadmium in this site. Furthermore, alimentary canal of both sexes (male and female) in polluted site showed higher concentrations of cadmium than testis and ovary.

Table 2. Concentration of cadmium (µg/g dry weight) in alimentary canal and gonads of both sexes relating to its concentration in soil samples at the selected sites

	Soil sample	Alimentary canal ♂	Alimentary canal ♀	Testis	Ovary
Reference site	4.80	2.96 ± 0.56	0.70 ± 0.26	1.85 ± 0.23	1.35 ± 2.04
Polluted site	59.0	30.21*±1.62	16.53* ±1.15	26.59*±1.31	12.85* ±1.43

Values are expressed as means ± standard errors.

(*) Differ significantly as compared with reference (P<0.05).

4.3. Biochemical results

In the present study, the results showed variation in the biochemical parameters of reproductive organs and alimentary canal of both sexes of the studied insects in response to pollution as follow:

4.3.1. Lipid peroxidation and glutathione content

Table 3 elucidated that there was a significant increase in the TBARS (index of lipid peroxidation) level of gonads and alimentary canal of both sexes in the studied insects collected from the polluted site when compared with that of reference group (p <0.05).

On the other hand, Table 3 revealed that the GSH content was significantly decreased in theses organs of the insects that collected from the polluted site when compared with that of reference one (P < 0.05).

Table 3. Thiobarbituric Acid Reactive Substances (TBARS) levels and Glutathione (GSH) content in gonads and alimentary canal of both sexes of insects

Values are expressed as means ± standard errors.

	TBARS (nmol/mg tissue)		GSH (mmol/mg.tissue)	
Site	Reference	Polluted	Reference	Polluted
Ovary	5.33 ±0.47	18.16*±2.42	23.13 ±1.59	14.23*±1.27
Testis	5.18 ±0.52	18.06*±1.52	21.75 ±1.56	12.50*± 1.3
Alimentary canal ♂	4.19 ± 0.34	11.31* ±1.38	27.88 ±3.64	20.06*±1.66
Alimentary canal ♀	4.68 ±0.39	9.25* ±0.72	27.88± 3.12	18.65*± 1.3

(*) Differ significantly as compared with reference (P<0.05).

ISSN 2347-6893

4.3.2. Antioxidant enzyme activities

Table 4 revealed that there was a significant reduction in the activity of the measured antioxidant enzymes (GST,CAT and SOD, respectively) of gonads and alimentary canal of both sexes in the studied insects collected from the polluted site when compared with that of the reference one ($p < 0.05$).

Table 4. Glutathione-S-transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) activities in gonads and alimentary canal of both sexes of insects.

Values are expressed as means ± standard errors.

(*) Differ significantly as compared with reference (P<0.05).

	GST (mU/mg protein)		CAT (mU/mg.protein)		SOD (mU/mg.protein)	
Site	Reference	Polluted	Reference	Polluted	Reference	Polluted
Ovary	11.2 ± 0.58	8.03*± 0.44	60.25± 3.19	44.63*±2.26	61.13±3.62	30.13*±3.18
Testis	11.50 ± 0.45	6.84* ± 4.82	62.62± 2.48	44.25*± 2.38	72.88± 4.59	33*± 2.46
Alimentary canal ♂	11.45 ±0.36	9.44*± 0.52	62.75 ±3.74	46.38* ±3.55	61.25 ±4.03	43.38*± 3.22
Alimentary canal ♀	11.86± 0.65	8.68*±0.58	59.88 ±2.73	41.63*± 3.02	60.13 ±4.28	36*± 3.6

4.3.3. AST and ALT enzymatic activities and total protein content

Table 5 depicted the results of AST and ALT enzymatic activities and total protein content of reproductive organs and alimentary canal of both sexes in the studied insects. The data revealed a significant increase in the enzymatic activities of AST and ALT in gonads and alimentary canal of both sexes in the studied species collected from the polluted site when compared with that of reference one ($p<0.05$). In addition a significant decrease in the total protein content was observed in gonads and alimentary canal of both sexes in the studied species collected from the polluted site when compared with that of reference one($p<0.05$).

Table 5. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) activities and total protein concentration levels in gonads and alimentary canal of both sexes of insects.

Values are expressed as means ± standard errors.

(*) Differ significantly as compared with reference (P<0.05).

	AST (mU/mg protein)		ALT (mU/mg.protein)		Total Protein Content (mg/ g.tissue)	
Site	Reference	Polluted	Reference	Polluted	Reference	Polluted
Ovary	134.63± 6.11	261.50*±11.49	68 ± 5.04	168.75*±9.61	148.63±12.74	78.38*±10.85
Testis	117.50 ±5.38	239*±16.82	67.5 ± 4.80	170.63*±10.04	153.25 ±12.46	90.24*±5.60
Alimentary canal ♂	114.38±7.64	222.50*±13.66	59.88 ±5.97	136.38*±9.33	132.63± 10.66	122.88* ±3.85
Alimentary canal ♀	124.13± 6.08	183.88* ±13.88	67.38± 3.50	126.63*±9.15	131.75± 3.54	113.50*±3.48

5. DISCUSSION

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. [21] The toxic action of cadmium causes oxidative damage to DNA, proteins and lipids. [22] Glutathione is the non-enzymatic radical scavenger, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes.[23] The present results indicated considerable decrease in GSH content in both testis and ovary in addition to the male and female alimentary canal of the studied species in response to pollution. The reduction of GSH content may be due to their consumption in the scavenging free radicals probably generated by heavy metals. Most studies on the relationship between cellular glutathione level and metal toxicity concluded that glutathione has a protective function against metal-induced toxicity.[24]During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulphide compound, oxidized glutathione.[25]As a result of GSH content becomes depleted and GSSG accumulates, thus indicating oxidative stress.

Elevation of LPO in both testis and ovary in addition to the male and female alimentary canal of the studied species collected from polluted site, as evidenced by the increased production of MDA in the present study. Several investigators showed that cadmium pollution increases MDA level in many insects.[26,27] The increased level of MDA may be due to the highly ROS, especially HO·, act on an unsaturated fatty acids of phospholipids components of membranes to produce MDA, a lipid peroxidation product.[28]

The present results revealed a significant decrease in GST, SOD and CAT activities in testis, ovary and in the male and female alimentary canal of insects collected from polluted site. Both enhancement and inhibition have been reported for the activity of antioxidant enzymes such as SOD, CAT or GST, depending on the metal levels, form and period of exposure, and species.[29,30]GST in insects represents an important line of defense against free radicals and may reflect the adaptation of insects to exposure with high concentrations of toxic chemicals.[31] The cumulative effect of Cd can inhibit the activity of glutathione-dependent enzymes including GST, through the inhibition γ-glutamylcysteine synthetase and glutathione biosynthesis.[32]There is closely relation between GSH content in the organisms and the activity of some enzymes, such as GST activity and GPx. Consequently the decrease in GSH content leads to reduction in GST in response to pollution. The superoxide radicals by themselves or after their transformation to H2O2 cause an oxidation of the cysteine in the enzyme and decrease SOD activity.[33] Cadmium shows chemical similarities to zinc and may affect the properties of zinc containing enzymes. The production of reactive oxygen by Cd is in part due to the inhibition of Cu/Zn SOD by replacing zinc with cadmium. The present finding agreed with Augustyniak et al. who concluded that cadmium reduced the activity of CAT in C. brunneus.[34] The decline in the CAT activity may be due to reducing the conversion of O2· - to H2O2 by SOD, which then leads to the accumulation of O2· -. Thus there is a certain relationship between CAT and SOD because SOD can convert the free O2· - to H2O2, which is then eliminated by CAT.[35]

The present results exhibited a significant increase of ALT and AST enzymatic activity as well as a significant decrease of total protein content in testis, ovary and the male and female alimentary canal. Several investigators also reported that heavy metal intoxication showed a significant increase in AST and ALT activities in the liver tissue of animals.[36,37]The increased in ALT and AST activities after exposure to pollution may be due to the degradation of proteins by proteolysis results in an increased amino acid pool and the prevalence of pathological conditions in the organ systems of an animal can also suppress protein synthetic capabilities, which in turn elevate the free amino acid concentration. The increased free amino acids may have been fed into the TCA cycle as keto acids by transdeamination since the activities of transaminases (ALT and AST) are also increased under pollutant stress.[38] Our results coincide with several investigators who reported the decline in total protein content in response to pollution.[38,39] It is known that proteins are biological compounds which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. Also they play a major role in the synthesis of microsomal detoxifying enzymes and help to detoxify the toxicants when entering into the animals. Consequently, the decrease of the total protein may reflect the decrease in the enzymatic activities of some enzymes in our study such as GST, GPX, SOD, 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 TCA cycle as α keto acid; they will help to supply energy for the insect. [40] It could be concluded from the present study that it is possible to use terrestrial insects especially tenebrionid beetles as sensitive indicators for environmental stresses by focusing on the analytical changes of biochemical parameters disturbed by environmental pollution.

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