The integration between insect biomonitoring study and GIS study to assess the pollution impacts as oxidative stress parameters within Abu-Zaabal industrial area

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Abstract

Sulphates, phosphates and heavy metals such as lead, cadmium and ferrous are considered as a serious environmental pollutant due to their ability to persistent within the environment and therefore case a serious damage to various ecosystem components.

This study aimed to develop and assess the integration models between biomonitoring process in form of oxidative stress components and a Geographic Information System (GIS), to evaluate the pollutants exposure and their effects on surrounding biomonitoring agent *Crocothemis erthrea* with respect to using a control study area . The biomonitoring processes were done using colorimetric analysis in form of oxidative stress parameters (including the activity of glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione-s-transferase (GST)) in brain, thoracic muscles, and gut of male and female *C. erthrea* collected from different studied areas.

The results showed that the activity of GR was increased in female than male and the increasing levels occurred along decreasing the distance from pollution source in male and female insects. However, there were a fluctuation in the activity of GPx in male insects, and GST in male and female insect along increasing the distance from pollution source with the highest value of 3x-fold and 4x fold, respectively, with respect to control.

Our study demonstrated the ability of to combine the biomonitoring-GIS modeling to assess the environmental pollutants and predict the deleterious effect of these pollutants on living organisms inform of oxidative stress issues.

Keywords: Environmental Pollutants; Heavy Metals; Oxidative Stress; Crocothemis Erthrea; GIS

Highlights

- We assessed the oxidative stress parameters to evaluate the biomonitoring potential of *Crocothemis erthrea* to environmental pollutants.
- Biomonitoring were evaluated in brain, thoracic muscles, and gut of male and female insect.
- The results of antioxidant responses approved the ability to using these oxidative stress parameters to quantify the deleterious effect of pollutants.

Introduction

The main air pollutants concentration emitted in Abu-zaabal industrial area are around 130 mg/m^3 nitrogen dioxide (NO_2), and 110 mg/m^3 smoke (Fig. 1a and b). Transport and deposition of such pollutants may have a hazardous effect on the environment, particularly air, soil, plant, and water (Kassir et al., 2012).

Environmental stress primarily increases the production of reactive oxygen species (ROS) in organisms. In aerobic cells, ROS are produced from molecular oxygen as result of normal cellular metabolism (Hermes-Lima, 2004; Sena and Chandel, 2012). Exogenous sources, such as fertilizer industries one of the main exogenous sources that can directly or indirectly influence the level of ROS in cells of different organisms (Amado et al., 2006; Dos Anjos et al., 2011). The three major ROS that are of physiological significance are superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Equation 1-5) (Gilbert 2000).

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad (1)$$

$$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet} \quad (2)$$

$$HO_2^{\bullet} + e^- \rightarrow H^+ + H_2O_2 \quad (3)$$

$$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^- \quad (4)$$

$$^{\bullet}OH + e^- \xrightarrow{H^+} H_2O \quad (5)$$

When ROS exceed normal level, lead to oxidative stress causing serious damage to macromolecules in living organisms, including DNA damage, protein carbonylation, lipid peroxidation, and enzyme inactivation (Halliwell and Gutteridge, 1984; Abdelfattah et al., 2017).

Protection against xenobiotics, including ROS-mediated environmental pollutants, can be realized by two main mechanisms: (i) the avoidance of stress, which cannot be achieved by organisms living in polluted areas or (ii) the intensification of the antioxidative defense of the organism (Migula et al., 2004). Antioxidants response - which includes enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (GST) - are supposed to be important indicators of oxidative stress (Livingstone, 2001; Lushchak, 2011; Abdelfattah et al., 2021) (Equation 10-15).

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (10)$$

$$2H_2O_2 \xrightarrow{CAT} H_2O + O_2 \quad (11)$$

$$H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG \quad (12)$$

$$LOOH + 2GSH \xrightarrow{GPx} LOH + H_2O + GSSG \quad (13)$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + 2NADP^+ \quad (14)$$

$$LOOH \mid 2GSH \xrightarrow{GST} LOH \mid H_2O \mid GSSG \quad (15)$$

Insects common in terrestrial and aquatic ecosystems, such as grasshoppers and dragonflies, can be seen, as sensitive to environmental changes. They can be considered as an interesting subject of ecotoxicological research, and a biomonitor of environmental pollutants, including air pollutants, and heavy metals, near an industrial region (Chen et al., 2005; Azam et al., 2015; Abdelfattah et al., 2021). One example of these insects is *C. erthrea* that used in ecotoxicological research, mainly to monitor the impact of pollutants due to high sensitivity to all changes of environmental parameters (Hassall and Thompson, 2008).

The present work was conducted to: Evaluate antioxidants levels enzymatic antioxidants such as (GPx, GR, and G-S-T) in brain, thoracic muscles, and gut of male, and female, of *C. erthrea* individuals inhabiting sites collected from different distances from pollution source and comparing to insects collected from control site. Consequently, the success of using integration between these biochemical parameters and GIS studies as biomonitoring for environmental pollution level.

Materials and methods

Study area

Insects were collected from four sites located at various distances from Abu-Zaabal industrial area

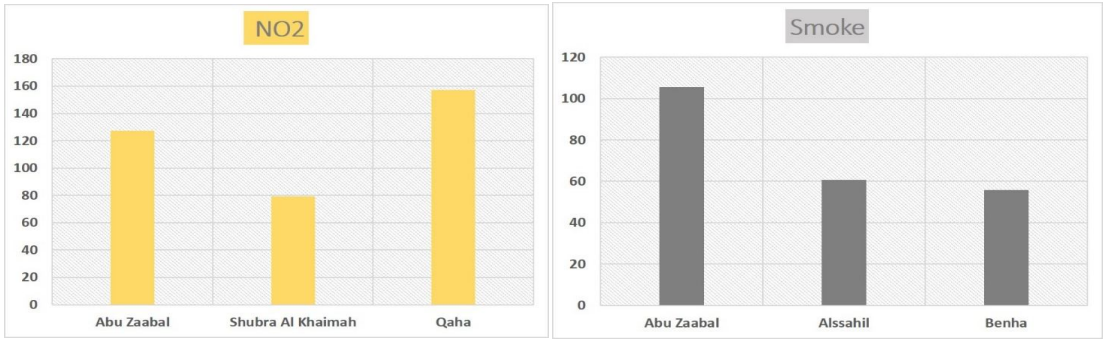


Figure1: showing the pollutants concentration, a) nitric oxide (N2O), b) smoke in the studied area

(the main contamination source). Specific conditions in the area allowed to set experimental plot along a pollution gradient. Three polluted sites (high, moderate and low)) of the cultivated spots of this area were located according to sampling collection sites (Fig. 2). Control site was established about 32 km from the source of pollution (Fig. 2). Data of air pollutants were obtained from Egyptian Environmental Affairs Agency, Ministry of Environment, Arab Republic of Egypt (Fig. 2). Lined polygon represents the fertilizer factory , yellow spots represent location of sampling sites

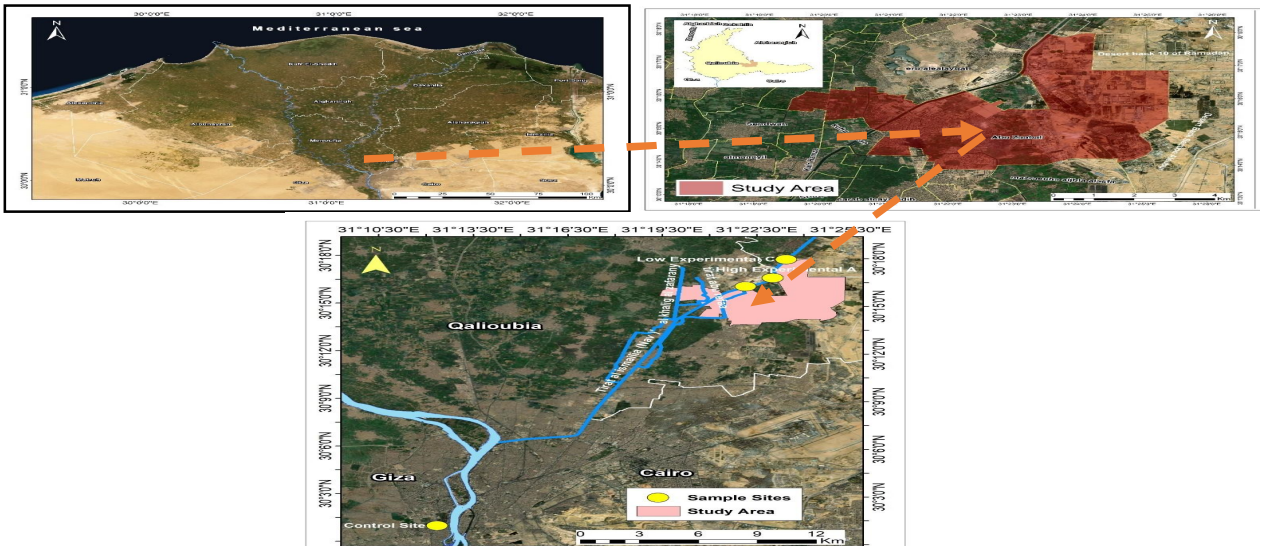


Figure 2: Map showing study area location.

Insect

Adult (males and females) of dragon flies, *Crocothemis erthrea* were collected with a sweep-net from four sites with different levels of pollution. Insects were transported to the laboratory in small 30 cm × 30 cm × 30 cm cages (approximately 25 insects per cage). Insects were dissected to isolate samples tissues (brain, thoracic muscles, and gut tissues) for further analysis and were stored at −20 °C until use. For each experimental group, 10 insects pool of male, and female of *C. erthrea* were prepared.

Biomonitoring assessment in form of oxidative stress assays

After dissection, the brain, thoracic muscles and gut tissues were homogenized (mortar, 10 strokes/30 s) in an ice-cold phosphate buffer (w/v ratio 1:4), the pH of the buffer was adjusted to pH= 7.0 using 2 M NaOH or 2 M HCl). The homogenates were centrifuged at 10,000×g for 30 min at 4 °C and the supernatants were used to measure the activities of the antioxidant enzymes.

The peroxidase activity was estimated according to the method described by Mazhoudi et al., (1997) with minor modifications. The reaction mixture containing 0.5 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.2 mL of 1% (m/v) guaiacol, 0.2 mL OF 0.4% (v/v) H_2O_2, and 0.1 mL of the enzyme extract from each sample tissue. Changes in the absorbance were measured at 470 nm over 3 min. POX activity was expressed as OD/min/mg protein.

In addition to, the activity of GR was determined according to Carlberg and Mannervik (1985) with minor modifications. The reaction mixture contained 0.5 mL of 2 mM oxidized glutathione (GSSG), 0.1 mL potassium phosphate buffer (50 mM, pH adjusted at 7.5 with 2 M HCl or NaOH), 0.2 mL of 3 mM DTNB, 0.1 mL of 2 mM NADPH, and 0.1 mL supernatant of the appropriate tissue. The absorbance was measured at 420 nm, and the GR activity was expressed as OD/µg protein/min.

Besides that, the activity of GST was determined according to method of Seyyedi et al. (2005) with minor modification. Reduced glutathione (GSH) and 1- chloro-2, 4-dinitrobenzene (CDNB) were used as substrates. The reaction mixture contained 100 µL of supernatant of the appropriate tissue sample, and 900 µL of the following mixture (882 µL PBS pH 7.0, 9 µL of 100 mM CDNB and 9 µL of 100 mM GSH). The absorbance was measured at 340 nm. G-S-T activity was expressed in OD/min/mg protein.

The total protein concentration of samples was determined spectrophotometrically according to the method of Bradford (1976), with Coomassie Brilliant Blue (COBB). The OD of the protein sample was measured at 595 nm against a blank of a tube containing distilled water instead of the protein sample. Bovine serum albumin (BSA) fraction V (Sigma-Aldrich) was used as a protein standard.

Statistical analysis

Non-parametric tests were carried out using the Mann–Whitney test for two median values and the k independent Kruskal–Wallis test for more than two median values. These statistical tests were done for antioxidants levels and expressed using median and quartile deviation (25th and 75th percentiles: P25 and P75).

Generalized Estimating Equation (GEE) was used to examine the effect of distance from the pollution source, types of tissues, sex and the interactions of these variables on antioxidants levels.

Correlations between the distance from pollution source and the biochemical results (antioxidants levels) were performed based on Pearson's regression analysis using multiple regression models.

Hierarchical Cluster Analysis (HACA) based on agglomerative statistics using Ward′s Method was calculated for antioxidants levels. The goal of HACA is to find possible clusters or groups among the observational units, based on level of similarities and differentiations (Azam et al., 2015). At each stage, the average similarity of the cluster is measured. While, the difference between each case within a cluster and that average similarity is calculated.

All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

Results

The relative levels of antioxidant enzymes in males, and females, and 5th instar of *Crocothemis erthrea* are shown in Fig.4-6. The activity of glutathione reductase (GR) in brain of male and female insects collected from control site was significantly the lowest value along experimental results. While, the activity of GR in gut of female's insects collected from moderate site was significantly higher than control site (Fig. 4).

The results showed that in control site, there was a significantly difference of GPx activity among brain, and thoracic muscles tissue in males and female insect ($p< 0.05$). Also, there was a significant difference among males and female insect in brain tissue of insect at low polluted site ($p> 0.05$). The highest value of GPx activity, occurred in the gut tissues of male insects collected from moderate polluted site (Fig.5). However, the highest value of GR activity occurred in the thoracic muscles of female insects (Fig. 4).

The activity of glutathione-s-transferase (GST) in brain, thoracic muscles, and gut tissues of male, and female insects showed a different pattern which involved a fluctuation activity of this enzymes along the distance away from pollution source (Fig. 6). However, in the tissues of males, and females, collected from the low polluted site, the level of G-S-T activity revealed a significant increasing effect compared to individuals from the control site (Fig. 6).

The concentration of enzymatic activity of all antioxidant enzymes (GPx, GR, and G-S-T) was significantly difference compared with control value. The correlation between distance of sampling sites from the pollution source and antioxidants response (enzymatic antioxidants) in *c. erythrea* were negatively or positively correlated which depend on the role of each antioxidant (Table 2).

A cluster analysis using Ward's method revealed slightly dissimilar patterns for males, and females however, the similar general tendency (Fig 7 and 8). The level of enzymatic response (GPx, GR, and G-S-T) was highly similar in brain of males, and female insects. Also, there was a separate cluster between control area and high polluted area in both thoracic muscles and gut tissues of male insects (Fig. 7). However, the cluster loop occurred between low, moderate and high polluted area in both thoracic muscles and gut tissues of female insects (Fig. 8).

The interaction analysis using GEE showed a significant influence of distance, sex, tissues, and intercept on all enzymatic antioxidants' response (GPx, GR, and GST) (*P value* <0.05) (Table 1).

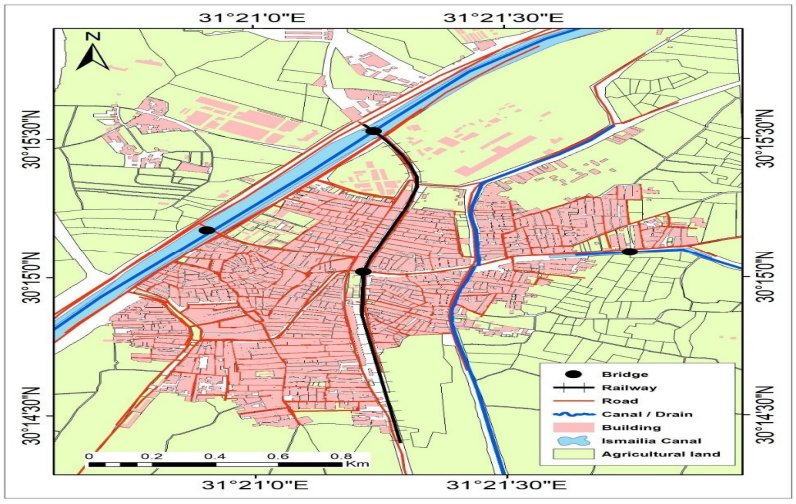
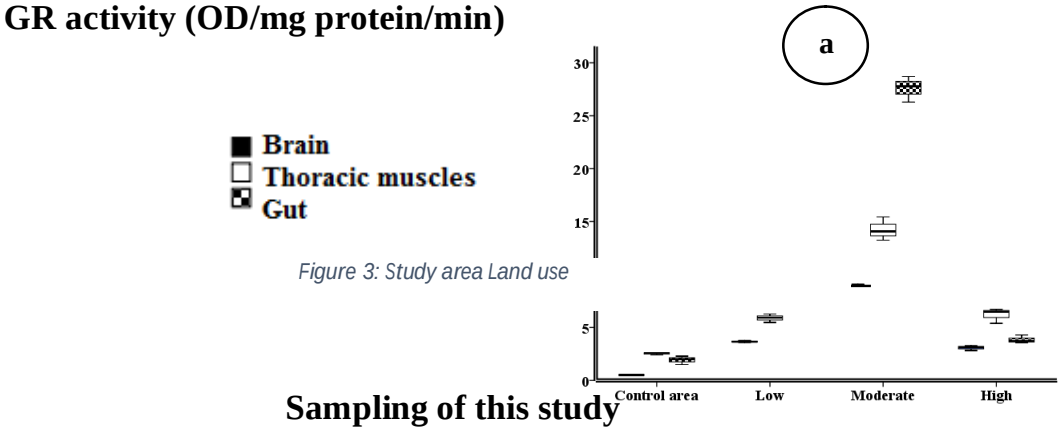


Figure 3: Study area Land use



Sampling of this study

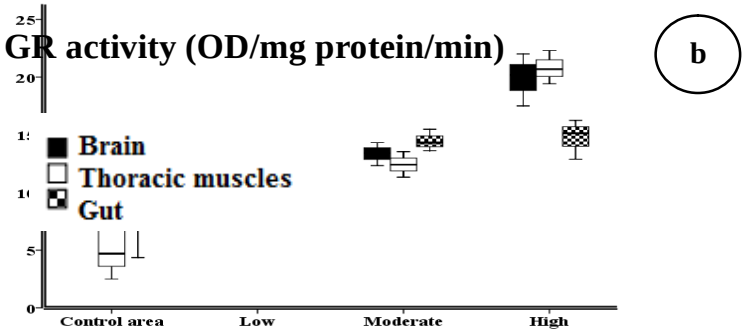


Fig. 4. Biomonitoring analysis, inform of glutathione reductase (GR) activity, expressed as OD/mg protein/ min, Of male (a) and female (b) insect, *Crocothemis erthrea* expressed as median, P25th and P75th in different experimental

Sampling of this study

Tissues brain, thoracic muscles, and gut insect tissues, collected from different experimental area (control area, low, Moderate, and high polluted area).

Mean values marked with same small letter means that there aren't significantly different among control And polluted area (low, moderate, and high) in each time period and insect tissue alone, (Kruskal-Wallis revealed, $p > 0.05$).

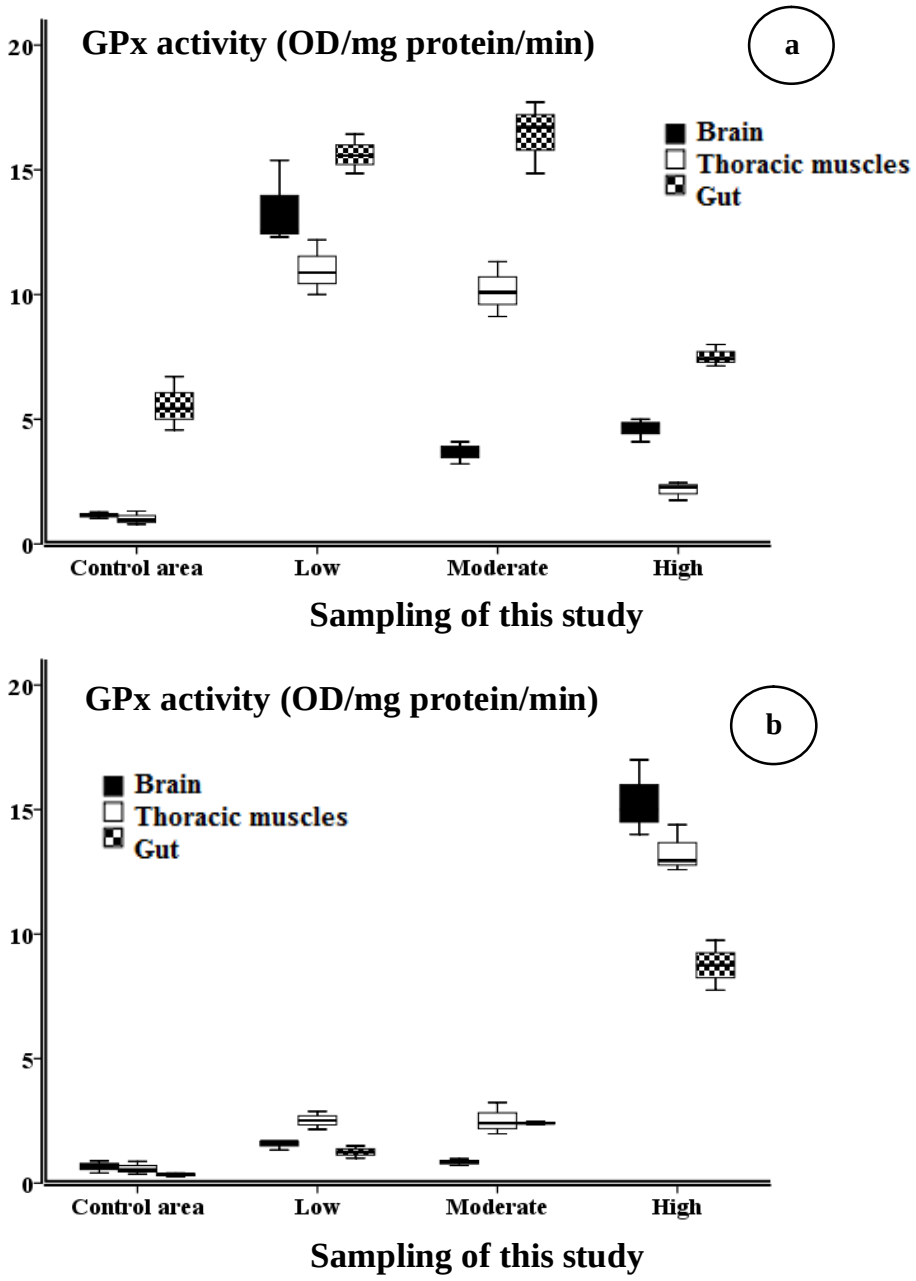


Fig. 5. Biomonitoring analysis, inform of glutathione peroxidase (GPx) activity, expressed as OD/mg protein/ min, of male (a) and female (b) insect, *Crocothemis erthrea* expressed as median, P25th and P75th in different experimental tissues brain, thoracic muscles, and gut insect tissues, collected from different experimental area (control area, low, moderate, and high polluted area).

Mean values marked with same small letter means that there aren't significantly different among control and polluted area (low, moderate, and high) in each time period and insect tissue alone, (Kruskal-Wallis revealed, $p > 0.05$).

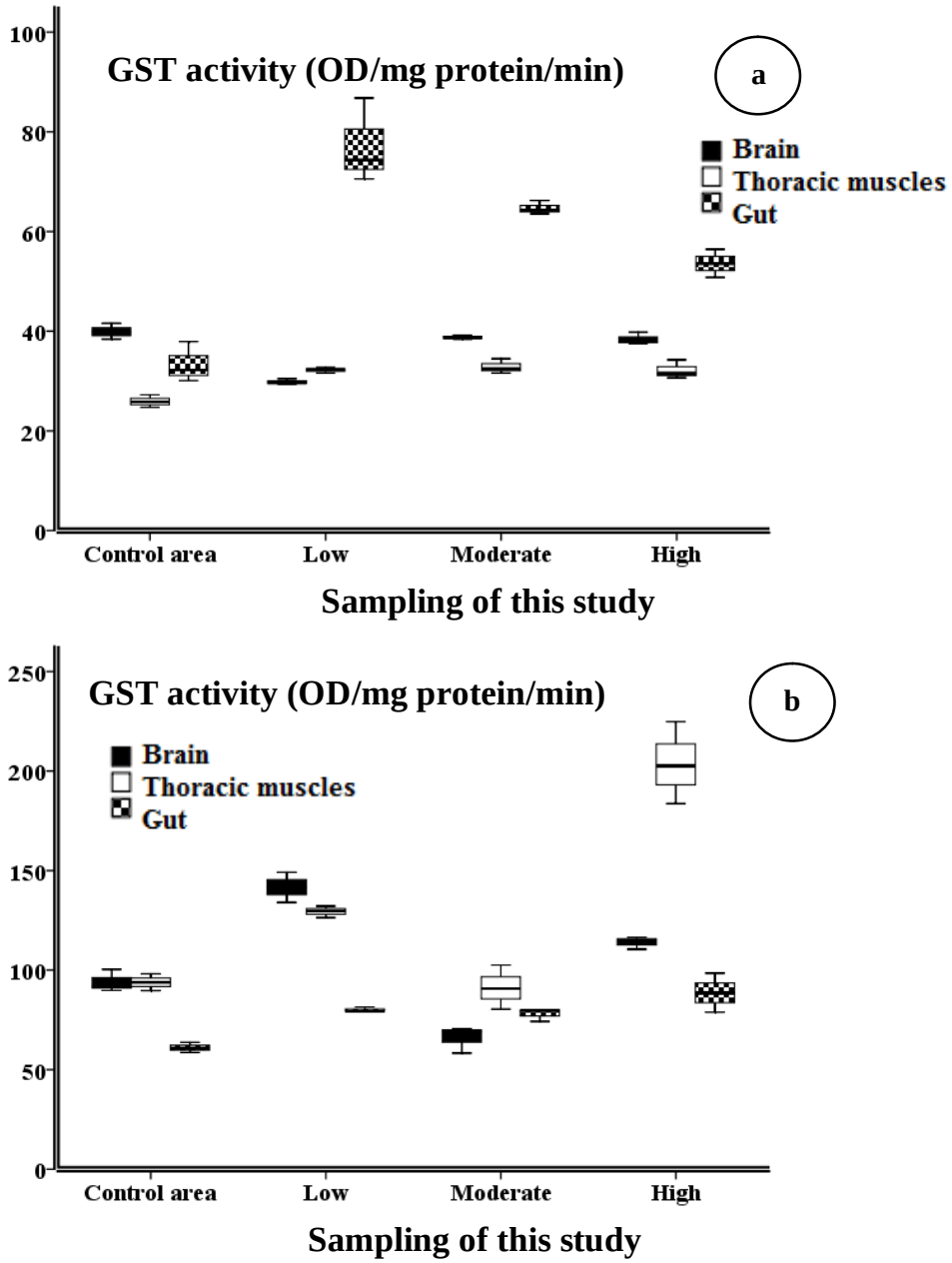


Fig. 6. Biomonitoring analysis, inform of glutathione-s-transferase (GST) activity, expressed as OD/mg protein/ min, of male (a) and female (b) insect, *Crocothemis erthrea* expressed as median, P25th and P75th in different experimental tissues brain, thoracic muscles, and gut insect tissues, collected from different experimental area (control area, low, moderate, and high polluted area).

Mean values marked with same small letter means that there aren't significantly different among control and polluted area (low, moderate, and high) in each time period and insect tissue alone, (Kruskal-Wallis revealed, $p > 0.05$).

Table 1. Generalized Estimating Equation to analyze the interactions among the distance from pollution source, types of tissues, sex on oxidative stress response (non-enzymatic antioxidants activity (GPx, GR, and G-S-T) in brain, thoracic muscles, and gut homogenates of male and female *Crocothemis erthrea* insect.

Item	Chi-square ($\varkappa^2$)	*df*	*P* value
	Tissue × Sex		
G-S-T	241	2	*< 0.0001*

GPX	219	2	< *0.0001*
GR	220	2	< *0.0001*
	Tissue × Pollutants concentration		
G-S-T	345	4	< *0.0001*
GPX	340	4	< *0.0001*
GR	341	4	< *0.0001*
	Pollutants concentration × Sex		
G-S-T	811	2	< *0.0001*
GPX	802	2	< *0.0001*
GR	803	2	< *0.0001*
	Tissue × Pollutants concentration × Sex		
G-S-T	187	4	< *0.0001*
GPX	165	4	< *0.0001*
GR	168	4	< *0.0001*
	Intercept		
G-S-T	10769	1	< *0.0001*
GPX	10147	1	< *0.0001*
GR	10175	1	< *0.0001*

Table 2. Pearson′s correlation coefficient among oxidative stress response, enzymatic response, (GPx, GR, and G-S-T) in brain, thoracic muscles, and gut homogenates of males, and females of *Crocothemis erthrea* and the distance from pollution source.

Enzymes	Sex	Tissue	Regression analysis	R^2	Type of equation	r
G-S-T	Male	Brain	$y= -5x^2+23x+12$	0.97	Polynomial	0.83**
		Thoracic muscles	$y= -0.7x^2+3x+30$	0.07		-0.15
		Gut	$y= 92\ e^{-0.18x}$	0.86	Exponential	-0.91**
	Female	Brain	$y= 62x^2-261+341$	0.97		-0.35
		Thoracic muscles	$y=75x^2-264x+318$	0.94		0.62
		Gut	$y=6x^2-21x+94$	0.46		0.51
GPx	Male	Brain	$y=5x^2-26x+33$	0.96		-0.80**
		Thoracic muscles	$y=-3x^2+10x+4$	0.96		-0.89**
		Gut	$y=-5x^2+15x+5$	0.95		-0.81**
	Female	Brain	$y=8x^2-24x+17$	0.98		0.83**
		Thoracic muscles	$y=5x^2-16x+13$	0.99	Polynomial	0.86**
		Gut	$y=3x^2-7x+5$	0.97		0.92**
GR	Male	Brain	$y=-6x^2+22x-13$	0.99		-0.09
		Thoracic muscles	$y=-8x^2+33x-19$	0.97		0.03
		Gut	$y=-21x^2+83x-53$	0.99		-0.18
	Female	Brain	$y=2x^2-3x+11$	0.91		0.93**
		Thoracic muscles	$y=5x^2-12x+20$	0.95		0.86**
		Gut	$y=-x^2+5x+8$	0.55		0.67**

* represents *p value* <0.05
** represents *p value* <0.01

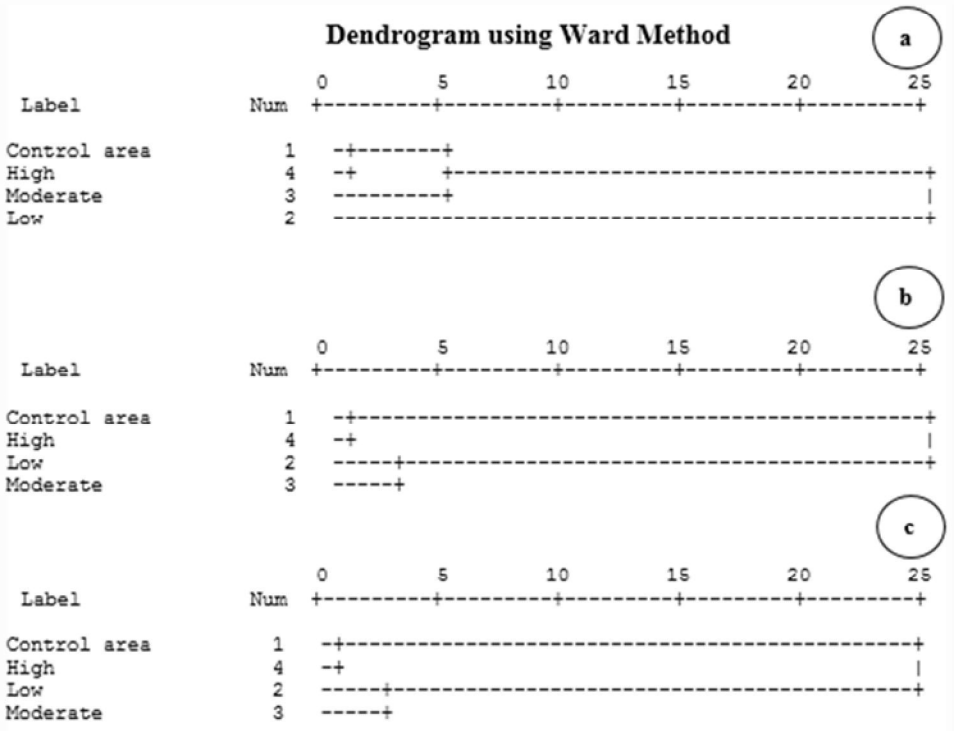


Fig. 7. Dendrogram of the cluster analysis (using Ward′s Method) applied for oxidative stress response, enzymatic response (GPx, GR, and G-S-T) in brain (a), thoracic muscles (b), and gut (c) homogenates of male of *Crocothemis erthrea*, which were collected at control site and polluted sites, located at different distances from pollution source.

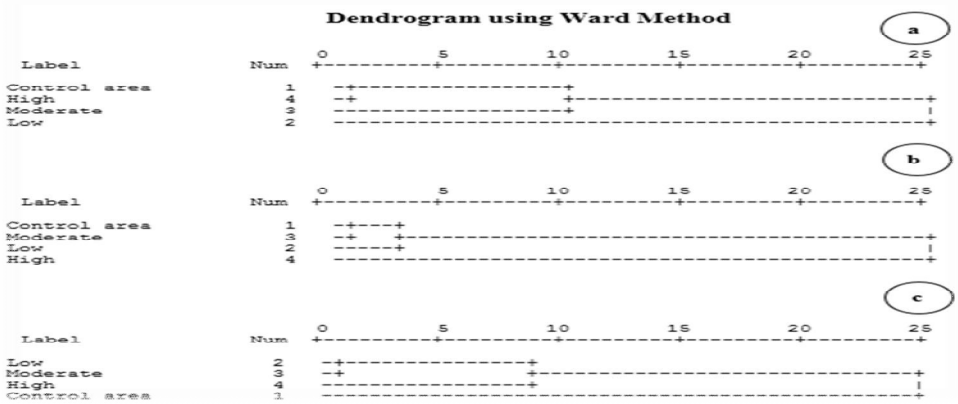


Fig. 8. Dendrogram of the cluster analysis (using Ward′s Method) applied for oxidative stress response, enzymatic response (GPx, GR, and G-S-T) in brain (a), thoracic muscles (b), and gut (c) homogenates of female of

Crocothemis erthrea, which were collected at control site and polluted sites, located at different distances from pollution source.

Discussion

In the present work, part of biomonitoring program designed to evaluate the biochemical changes in insect, *Crocothemis erthrea*, collected from different sites around pollution source in Abu-Zaabal industrial area. However, the air pollutants (smoke and nitric oxide) concentration were generally within national (Egyptian limits) and international standard limits (US EPA, 2014); there are a significant effect on oxidative stress parameters related to pollution gradients.

The obtained results revealed the information that proteins, which is the main source of enzymes are important targets of free radical attack in the cells (Lushchak, 2011), and thus the antioxidant defense, cellular function, and finally organism survival can be impaired. ROS are known to convert amino groups of proteins and thereby, change protein structure and function. The oxidative stress increased the number of modified carbonyl groups correlates with protein damage (Hermes-Lima, 2004). Also, ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins, and oxidation of specific amino acids and therefore lead to increase susceptibility to proteolysis by degradation of specific proteases (Kelly and Mudway, 2003; Abdelfattah et al., 2021). The oxidation of proteins leads physiologically to disruption of conformation and vital functions of protein molecules, including enzymes, and other regulatory functions of the cell (Korsloot et al., 2004; Birben et al., 2012; Yousef et al., 2017).

High level of ROS lead to increases the activity of antioxidant enzymes, such GPx, GR, and GST (Fig. 5-7). In *Oxya chinensis*, high concentrations of Cd acted directly, caused increase in reactive oxygen species, and changes in SOD, CAT, APOX, PPO, POX G-S-T, and GR activity (Shukla et al., 2017). The authors suggested that multiple mechanisms rather than a single mechanism may be responsible for the capacity of insects to resist cadmium. Moreover, they concluded that CAT has a strong detoxification function and play the most important role in limitation of the damaging effects of reactive oxygen species in *O. chinensis* injected with cadmium (Lijun et al. 2005).

The key role of GPx, GR, and GST in ROS scavenging, was studied (Yousef et al., 2017; Abdelfattah et al., 2021). APOX, and GPx catalyzes the reduction of lower limit of H_2O_2 levels with consumption of ascorbate as the reducing agent. Therefore, GPx activity depends exclusively on the availability of reduced ascorbate. Under normal conditions the cellular pool of ascorbate is kept in a reduced state by a set of enzymes, namely mono-dehydroascrobate reductase (MDAR) and dehydroascorbate reductase (DHAR) capable of using NAD(P)H to regenerate oxidized ascorbate (Farooqui and Farooqui, 2011). The present work confirmed the positive correlation between APOX activity in gut of both sexes, and the distance of insect collection sites from the pollution source. It revealed that the oxidative stress markers of gut homogenates in comparison to other tissues were frequently higher in insects collected from different experimental sites. This is probably because the gut of this insect is usually subjected to prooxidants ingested in food, as reported in other phytophagous insects (Ahmad, 1992; Felton and Summers, 1995; Krishnan and Kodrik, 2006; Abdelfattah et al., 2021).

The present result usually showed that the activity of antioxidant enzymes (GPx, GR, and GST) in female tissues were higher than male insect. (Fig. 5-7). It was suggested that the depletion of the antioxidant enzyme activity in male insect may be due to accumulation of heavy metals in male more than female, which lead to inactivate enzyme activity as result of structural changes of the antioxidant enzyme (Iszard ,1995; Wilczek et al. 2003; Yan et al., 2007). Also, Zhang et al. (2011) referred the depletion of antioxidant enzyme activity occurred due to decrease in protein synthesis which depend on aging (developmental stage).

Research conducted in this study suggests that significant differences of environmental stress marker levels in *c. erythrea* are not a direct result of the site of insect collection but depend on differences in contamination and pollutants concentration in environment among studied sites. Similar results and finding were observed by Ihechiluru et al., (2015), who found insignificant difference between oxidative stress markers and the sites of collections, however there were strong positive or negative overall correlations between heavy metal concentrations in insects and respective oxidative stress markers.

Acknowledgments

This study was done at the Entomology Department, Faculty of Science, Cairo University, Egypt.

Compliance with ethical standards

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Disclosure of Potential Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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