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Zeinab A El-Moaty
Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Dalia A Kheirallah
Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Doha A Elgendy
Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Correspondence
Zeinab A El-Moaty
Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Impact of Cement Dust on Some Biological Parameters of *Trachyderma hispida* (Coleoptera: Tenebrionidae) Inhabiting The Vicinity of a Cement Factory, Mariout region, Alexandria, Egypt

Zeinab A El-Moaty, Dalia A Kheirallah and Doha A Elgendy

Abstract
The objective of this study was to quantify the concentration of heavy metals (Cu, Pb, Cr, Zn, Cd, Co and Ni), levels of enzymes (AST and ALT), protein and nucleic acid (DNA and RNA) content in the ground beetles, *Trachyderma hispida* at two sampling sites. The selected sites that varying considerably in the load of air pollution, located in the Mariout region, west of Alexandria on the Mediterranean cost, Egypt. The first site (cement dust site) was near to the cement factory- Elhammam city. While the second one (reference site) was at Burg-ElArab city. Insects were sampled in two periods: breeding period (April-November), and non-breeding period (December-March).The data obtained from this study showed that the concentration of Cu and Ni in the soil and leaf litter were obviously high in both studied sites. In addition, six mean differences of heavy metals out of seven were proven to be highly significant in the cement dust than the reference site. No difference in levels of Cr in soil and leaf litter was detected in the two sites ($p>0.05$). Insects collected over the breeding and non-breeding periods contained variable concentration levels of most tested heavy metals in the hard exoskeleton than soft tissues of males as well as females in both cement dust and reference sites. A significant decline in enzyme activities in both males and females sampled from the two selected sites during the non-breeding period as compared to insects sampled during breeding period ($p>0.05$). A significant reduction in protein biosynthesis and transcriptional activity was recorded in insects collected from the cement dust site ($p<0.05$). So, it could be concluded that *T. hispida* is severely affected by pollutants in the environment, resulting in obvious changes in biochemical processes and cytogenetic parameters.

Keywords: ecotoxicology, ground beetles, heavy metals, air pollution

1. Introduction
Organisms may respond to environmental stressors either behaviorally or by means of biochemical and physiological mechanisms to compensate the stressors negative effects. Industrialization increases the problem of the environmental pollution (particularly air pollution) and become more difficult to be controlled. Cement dust is considered as a major source of toxic air emission. It contains many toxic substances such as calcium carbonate and sodium sulphate as well as heavy metals [1, 2]. Accumulated large quantities of heavy metals in the environment are potential risk to human and all other life forms [3- 5]. Biomonitoring studies involved the use of biological or molecular markers as indicators signaling events in organisms exposed to environmental pollutants. Therefore, biological monitoring (through analysis of cells, tissues or body fluids of exposed organisms) may lead to identification of potentially hazardous exposures before adverse effects appear [6].
The possibility of insects' inclusion in ecotoxicological investigations is a matter of increasing concern, due to their mobility and abundance [7- 8]. Ground beetles are often strongly specific to certain habitat type, have low inter-patch dispersal rates and are easy to collect in sufficiently large numbers to allow statistical analyses [9]. Consequently, they are excellent indicators of habitat quality and are widely used in biological surveys [10 -11].
Carabid population in a contaminated site showed significant elevation of metal concentrations as compared with population in the reference site [12]. Concentrations of Ni and Cu in the leaf beetle *Chrysomela lapponica* from the polluted site were 7.7 and 3.6 times, respectively higher than that in beetles from unpolluted habitats[13]. Moreover, there were correlations between the heavy metal concentrations in ground beetles and soil or leaf litter; positive for Pb and Cd

concentrations and negative for iron concentration [11] *Carbide beetles, Pterostichus oblongopunctatus*, larvae fed on Cd or Zn treatments grew significantly slower and had the lowest survival rate in respect to control [14]. Adults of this species responded to heavy metal pollution by decreasing the body size [15] or altering life-history parameters [16]. These results suggested that pollutants have negative effects on the development and subsequently fitness of this species.

Changes in enzyme concentration in cells should therefore reflect states of health of a living organism. Some enzymes exist free in the cellular fluid and others are contained in cellular structures such as the mitochondria and lysosomes. As long as the cell membrane is intact, such enzymes are contained within the cell and the level of these enzymes in extracellular fluid is extremely low. If cells are damaged in some way, the membrane becomes permeable or ruptures. The cell contents, including their enzymes complements, are then released into the extracellular fluid and the enzymes may increase suddenly to levels many times greater than normal.

Environmental stressors such as contaminants can affect insects at the molecular or sub cellular level and can be expressed as increase in enzyme activity or nucleic acid damage [17]. There are two enzymes showing the greatest diagnostic potential; Aspartate aminotransferase (AST or GOT) and Alanin aminotransferase (ALT or GPT) [18]. In this regards, Breem [19] found significant lower activities of AST and ALT in the aquatic insect *Sphaerodema urinator* subjected to lethal and sub lethal levels of lead nitrate and copper sulphate.

Proteins are the most important and abundant macromolecules in the living organisms and there are some indications that protein metabolism is affected by cadmium exposure in diverse arthropod species [20-21] Similarly, Jamil *et al*. [22] reported a drastic decline in protein content of the hyacinth weevil *Neochetina eichhorniae* fed on lead treated plants. In addition, Kheirallah *et al.* [23] observed significant decrease in total protein in the whole body of males and females of *S. urinator* inhabiting a highly polluted area of Lake Mariout, Alexandria compared with those collected from mildly polluted area of Lake Edko (Alexandria city).

The informational molecules of all living organisms are composed of Deoxyribonucleic acid (DNA). Toxicant effects may be studied by examining changes in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). However, with regard to environmental contaminants, few studies have been performed on insects. Feeding of hyacinth weevils *Neochetina eichhorniae* on lead treated plants *Eichhornia crassipes* inhibited nucleic acids DNA and RNA synthesis [22]. Moreover, Kheirallah *et al*. [23] observed significant decrease in the contents of RNA and DNA, RNA/ DNA and protein/ DNA ratios in the whole body of both sexes of *S. urinator* collected from heavily polluted sites of Lake Marioute (Alexandria city).

The current study focused on habitat-specific analysis of the coleopterous terrestrial insect *T. hispida* inhabiting two sites varying considerably in the load of air pollution. The present study uses a set of biochemical methods include (1) Determination of pollutant levels in soil and leaf litter of the selected sites;(2) Determination of heavy metals concentration in the hard exoskeleton and soft tissues of both sexes of the inspected insects during breeding and non-breeding periods;(3) Tracing the changes occurring in the concentrations of some biochemical constituents involving total proteins, levels of AST and ALT as well as determination of DNA and RNA of insects caught from the inspected sites.

2. Material and Methods

2.1 Study areas

The present study was carried out in the Mariout region which is a strip of land about 100 km long and 30 km wide west of Alexandria city on the Mediterranean cost, Egypt (Fig.1).Two uncultivated sites were chosen for sampling the studied species. These sites were (A) unpolluted site (reference site) at Burg El-Arab city, which is 53 km west of Alexandria and (B) polluted site (cement dust site) at El-Hammam city, which is 62 km west of Alexandria. These sites are occupied by many halophytic plant species. The cement dust area surveyed at El-Hammam city is 1 km downwind from a cement factory. This area showed signs of cement dust deposition on the vegetation as well as soil surface.

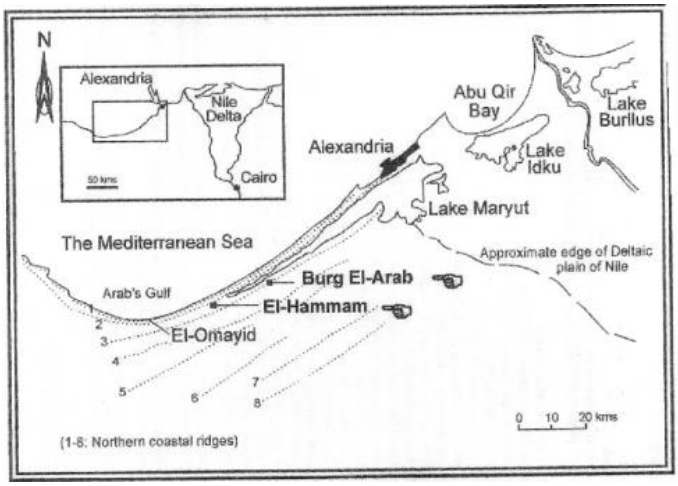


Fig 1: A map showing the studied region, the Mariout region, west of Alexandria, Mediterranean cost, Egypt

2. Specimens identification

Preliminary collections of coleopterous insects inhabiting the selected sites showed that *T. hispida* is one of the dominant adult species in the studied sites. The Specimens were identified (through personal communication) by the taxonomists Dr. A. Rolf (Department of entomology, California Academy of Sciences USA) as *T. hispida* (Forskal, 1775). The studied species belongs to Tenebrionid beetles (darkling beetles).

2.3 Sampling procedure

In the studied sites, the coleopterous insect *T. hispida* was confined to the spaces beneath rocks and canopy of the shrubs. Adults were available in early morning. Collected insects were sexed then maintained alive in native soil and plants in suitable jars until processing. Stimulatory with insect collection, soil samples at a depth of 30 cm below the surface were collected from the specific sampling sites. Soil samples were dried and passed through 0.2 mm sieve to eliminate gravel and debris. Insects were sampled in two periods; the first in breeding period (April-November), and the second in non-breeding period (December-March).

2.4 Heavy metal analysis

The determination of heavy metals Cu, Pb, Co, Zn, Cd, Cr and Ni concentration in sieved soil and leaf litter samples was carried out according to Loring and Rantala [24] using atomic absorption spectrophotometer (Berkin-Elmer model 2380) under the recommended conditions and detection limits (DL)

in the manual for each metal.

The concentrations of different heavy metals were determined in the hard exoskeleton and soft tissues of both sexes of studied insects and were evaluated by the method described by UNEP/FAO/IAEA/IOC (1984) (cited by El-Sikaily *et al.*) [25]. All data are presented as concentration per unit wet weight of the sample (as mg\g).

2.5 Biochemical analysis

Various biochemical parameters; enzyme levels of AST and ALT, Protein content and nucleic acid contents (RNA, DNA) were determined in the whole body of males and females collected from the inspected sites during breeding and non-breeding periods.

Levels of enzymes AST and ALT were assayed according to the method outlined by Reitman and Frankel [26], using available kits from Diamond Colorimetric. Protein concentration that assessed by using Biosystems kits, according to Gornall *et al.* [27], was measured in µg/100 mg tissue. Nucleic acid (RNA and DNA) contents were determined according to the method of Barron and Adelman [28]. Nucleic acid (RNA and DNA) content expressed as µg/100 mg tissue.

2.6 Statistical Analysis.

Statistical analysis was performed using the program SPSS [29]. Data were analyzed statistically by using t- test and One-way analysis of variance (ANOVA) [30] to determine the difference between the two studied sites.

3. Results

3.1 Bioaccumulation of heavy metals

The obtained data from the analysis of soil and leaf litter samples collected from the inspected sites showed that the concentration of Cu and Ni in the soil and leaf litter were obviously high in both cement dust and reference sites (Fig.2). Based on the t-test, significant differences were found for Cu and Ni with higher concentration in leaf litter than the soil in both sites; in cement dust site (for Cu t=29.23; p= 0.0012 and for Ni t=16.89; p= 0.0001) and in reference site (for Cu t=15.54; p=0.0041 and for Ni t= 13.55; p=0.0009). The studied sites appear to be rather dissimilar with respect to the concentration of the studied heavy metals. Statistical analysis for the mean differences of each metal revealed that six mean differences out of seven were proven to be highly significant in the cement dust site than the reference site. It is of considerable interest that no difference in levels of Cr in soil and leaf litter was detected in the two sites ($p>0.05$) (Table 1).

It is apparent for the concentrations of heavy metals in hard exoskeleton and soft tissues of both males and females collected from the inspected sites over the breeding and non-breeding periods that in all cases Cu and Ni recorded the highest concentrations among all tested heavy metals (Fig. 3). Males collected over the breeding period contained higher concentration of Pb, Zn and Ni in hard exoskeleton and soft tissues. While Cd and Ni were the most dominant in females. It was also noticed that during the non-breeding period Pb, Cd and Ni were the most dominant heavy metals in the soft tissues of females.

Statistical analysis showed that both studied periods there were significant differences in the concentration of Pb and Ni in hard exoskeleton of males, Pb, Cd and Ni in soft tissues of males, Cu, Cd and Ni in hard exoskeleton of females and Pb, Cd and Ni soft tissues of females between the two selected sites. On the other hand, between the two selected sites, there were insignificant differences in the concentration of Cr, Co and Zn in hard exoskeleton of males, soft tissues of males, hard exoskeleton of females, respectively as well as in the concentration of Co and Zn of soft tissues of females (Table 2).

Table 1: Statistical analysis (One way ANOVA) of the heavy metals concentrations (ug/g) in the soil and leaf litter collected from reference and cement dust sites.

	Heavy metals	One way ANOVA	
		F	P
Soil	Cu	288.0*	≤0.001
	Pb	69.588*	≤0.001
	Co	240.134*	≤0.001
	Zn	1943.327*	≤0.001
	Cd	1575.538*	≤0.001
	Cr	6.451	>0.05
	Ni	11.115*	≤0.05
Leaf litter	Cu	257.143*	≤0.001
	Pb	10.526*	≤0.05
	Co	58.790*	≤0.05
	Zn	52.563*	≤0.05
	Cd	34.031*	≤0.05
	Cr	1.937	>0.05
	Ni	59.645*	≤0.05

*: Statistically significant at $p \leq 0.05$

Table 2: One way analysis of variance of the heavy metals concentrations (ug/g) in the hard exoskeleton and soft tissues of males and females *T. hispida* collected during the breeding and non-breeding seasons from reference and cement dust sites.

Sex	Tissue	Heavy metals	Breeding season		Non-breeding season	
			F	p	F	p
Male	Hard Exoskeleton	Cu	0.619	>0.05	95.066*	≤ 0.001
		Pb	38.545*	≤0.01	17.483*	≤ 0.05
		Co	14.582*	≤ 0.05	0.532	>0.05
		Zn	11.332*	≤ 0.05	0.138	> 0.05
		Cd	0.628	> 0.05	40.612*	≤ 0.01
		Cr	1.257	> 0.05	0.391	>0.05
		Ni	31.895*	≤ 0.01	9.570*	≤ 0.05
	Soft Tissues	Cu	0.001	>0.05	195.174*	≤ 0.001
		Pb	12.277*	≤ 0.05	37.500*	≤ 0.01
		Co	4.355	>0.05	0.118	>0.05
		Zn	219.800*	≤0.001	4.028	>0.05
		Cd	105.022*	≤0.001	73.960*	≤ 0.001
		Cr	5.796	> 0.05	25.474*	≤ 0.01
		Ni	33.108*	≤ 0.01	88.493*	≤ 0.001
Female	Hard Exoskeleton	Cu	24.898*	≤ 0.01	11.546*	≤ 0.05
		Pb	5.220	> 0.05	24.014*	≤ 0.01
		Co	11.225*	≤ 0.05	2.012	>0.05
		Zn	3.880	> 0.05	4.765	>0.05
		Cd	9.236*	≤ 0.05	53.635*	≤ 0.01
		Cr	3.765	> 0.05	26.000*	≤ 0.01
		Ni	43.655*	≤ 0.01	25.350*	≤ 0.01
	Soft Tissues	Cu	0.175	>0.05	19.209*	≤ 0.05
		Pb	16.078*	≤ 0.05	52.364*	≤ 0.01
		Co	4.923	>0.05	1.923	>0.05
		Zn	0.116	>0.05	0.735	>0.05
		Cd	22.893*	≤ 0.01	55.385*	≤ 0.01
		Cr	3.786	>0.05	61.364*	≤ 0.001
		Ni	12.005*	≤ 0.05	132.787*	≤ 0.001

*: Statistically significant at p ≤ 0.05

Data was expressed as (Mean ± SEM)

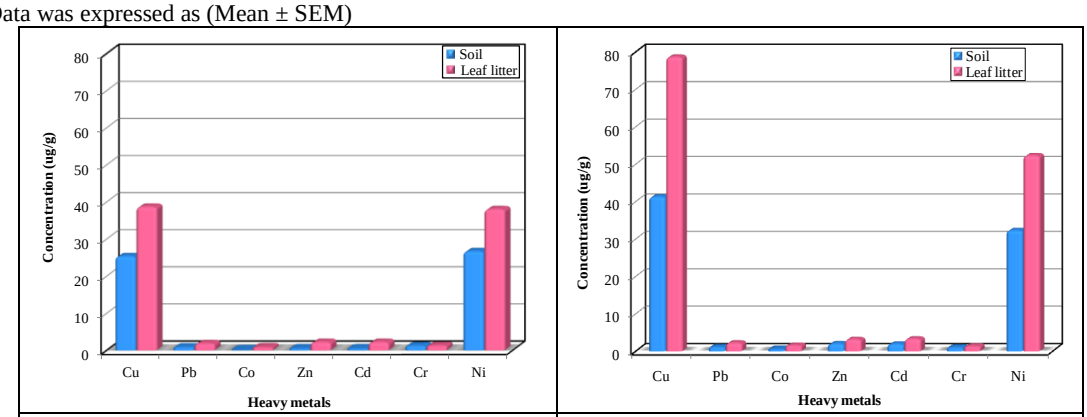
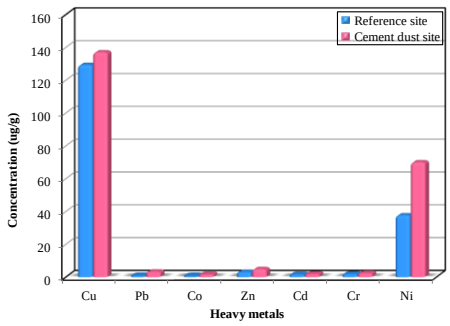
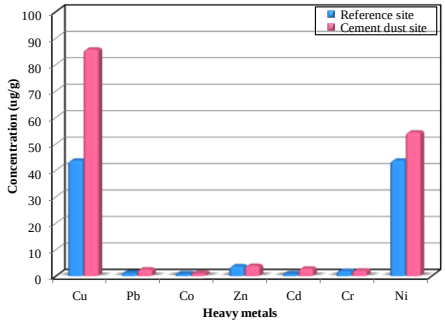


Fig (2): Heavy metals concentrations (ug/g) in the soil and leaf litter sampled from both reference and cement dust sites.

		Breeding season	Non-breeding season
Male	**Hard Exoskeleton**		

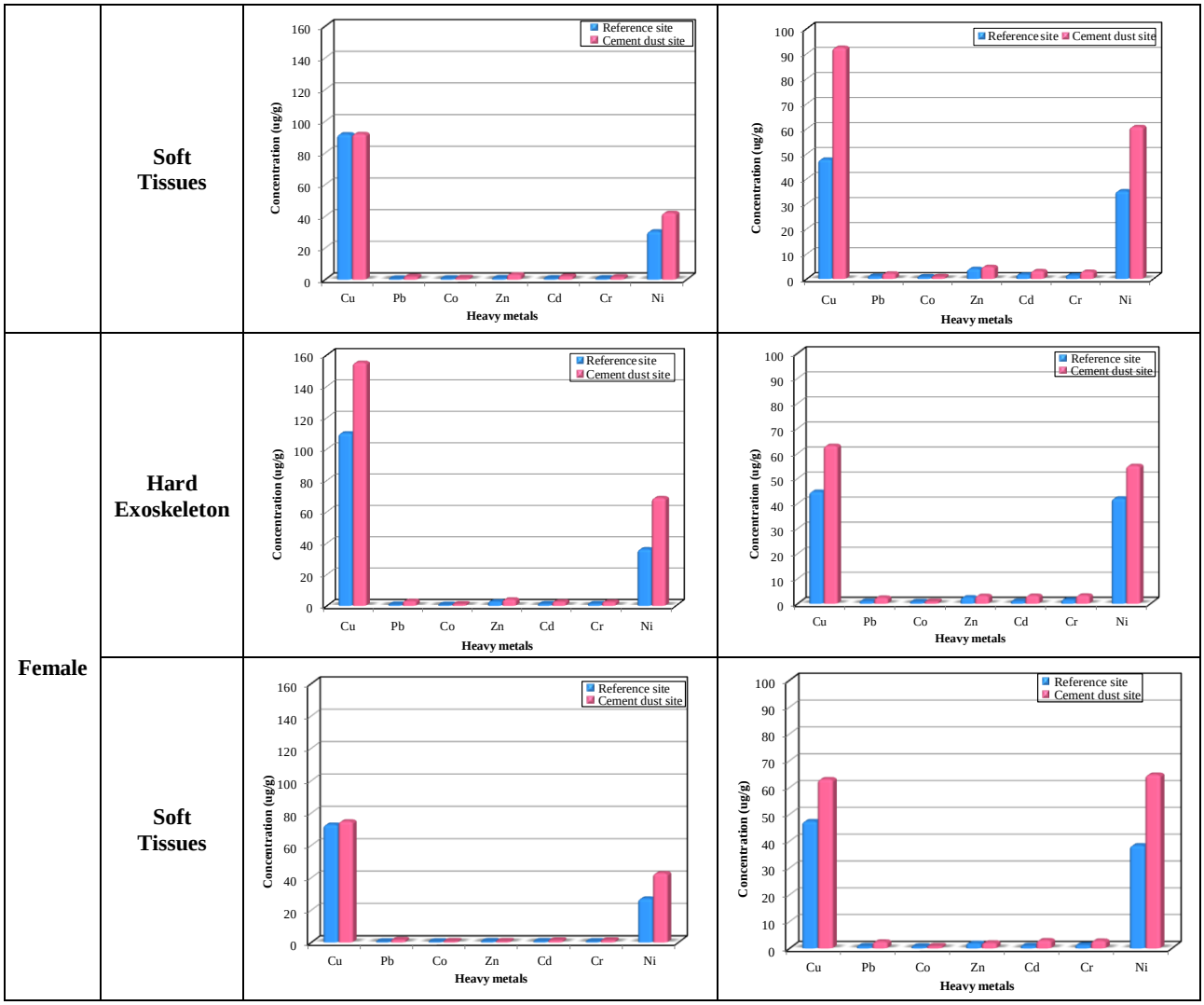


Fig (3): Heavy metals concentrations (ug/g) in the hard exoskeleton and soft tissues of males and females *T. hispida* collected during the breeding and non-breeding periods from reference and cement dust sites.

3.2 Biochemical assessment

There were significant differences in the levels of AST and ALT content among all groups of insects collected during breeding and non-breeding periods from cement dust site as compared to the reference site (Table 3). The data also indicate a decline in enzymes level both in males and females sampled from the two selected sites during the non-breeding period as compared to insects sampled during breeding period (Fig. 4).

It is clear that each unit of the DNA in females and males sampled during breeding period, from reference site was capable to transcribe 0.70, 0.50 units, respectively of RNA and this led to synthesize 3.45 and 4.77, respectively of protein. Whereas at cement dust site, a significant reduction in transcriptional activity (0.36, and 0.46 in females and males, respectively) and protein biosynthesis (1.59, and 2.12 in females and males, respectively) were detected. A similar trend was noted during non-breeding period where the values of transcriptional activity of females and males sampled at reference site were 0.53 and 0.60, respectively of RNA and this led to synthesize 4.07 and 4.61, respectively of protein. On the other hand, at cement dust site, a significant reduction in transcriptional activity (0.50 and 0.53 for females and males, respectively) and protein biosynthesis (1.82, 2.38 for females and males, respectively) was recorded (Fig. 5).

The statistical analysis displayed significant difference in protein and nucleic acid content amongst all groups during the studied periods, being highest in insects collected from the reference site and lowest in those collected from the cement dust site. Moreover, the levels of protein and nucleic acid content in males and females sampled from the inspected sites showed an obvious alteration among the studied periods being highest in most cases during the breeding period (Table 4). Statistical analysis between means (F-test) revealed significant ($p<0.05$) lower content of protein and nucleic acids in both sexes sampled during the studied periods from cement dust site as compared to the reference site except for DNA of the male collected during the non-breeding period. Comparing the data of protein and nucleic acid content of males and females of each location between the two studied periods, ANOVA showed that there was a significant decrease ($p<0.05$) in all tested parameters at each site during breeding and non-breeding period (Table 4).

Table 3: One way analysis of variance of the activities of AST and ALT in body of males and females *T. hispida* sampled during the breeding and non-breeding seasons from the inspected sites.

Season	Sex	Enzymes	One way ANOVA	
			F	p
Breeding	Males	AST	50.704*	≤0.05
		ALT	29.268*	≤0.05
	Females	AST	79.048*	≤ 0.01
		ALT	171.738*	≤0.001
Non-breeding	Males	AST	93.011*	≤ 0.001
		ALT	312.050*	≤ 0.001
	Females	AST	150.645*	≤ 0.001
		ALT	460.800*	≤ 0.001

F: test (ANOVA)
*: Statistically significant at $p \leq 0.05$

Table 4: One way ANOVA Protein and nucleic acid (RNA and DNA) content, RNA/DNA and Protein/DNA (mg/g) in body homogenate of male and female *T. hispida* sampled during the breeding period from reference and cement dust sites.

Sex	Macromolecules	Breeding period		Non-breeding period	
		F	p	F	p
Male	Protein	121.133*	≤ 0.001	290.457*	≤ 0.001
	RNA	39.052*	≤ 0.01	12.094*	≤ 0.05
	DNA	38.473*	≤ 0.01	5.593	>0.05
	RNA/DNA	2.431	>0.05	2.043	>0.05
	Protein/DNA	78.059*	≤ 0.001	51.764*	≤ 0.01
Female	Protein	300.052*	≤ 0.001	494.815*	≤ 0.001
	RNA	26.176*	≤ 0.01	137.317*	≤ 0.001
	DNA	12.816*	≤ 0.05	44.695*	≤ 0.01
	RNA/DNA	60.410	≤ 0.001	1.015	>0.05
	Protein/DNA	74.880*	≤ 0.001	304.413*	≤ 0.001

*: Statistically significant at $p \leq 0.05$

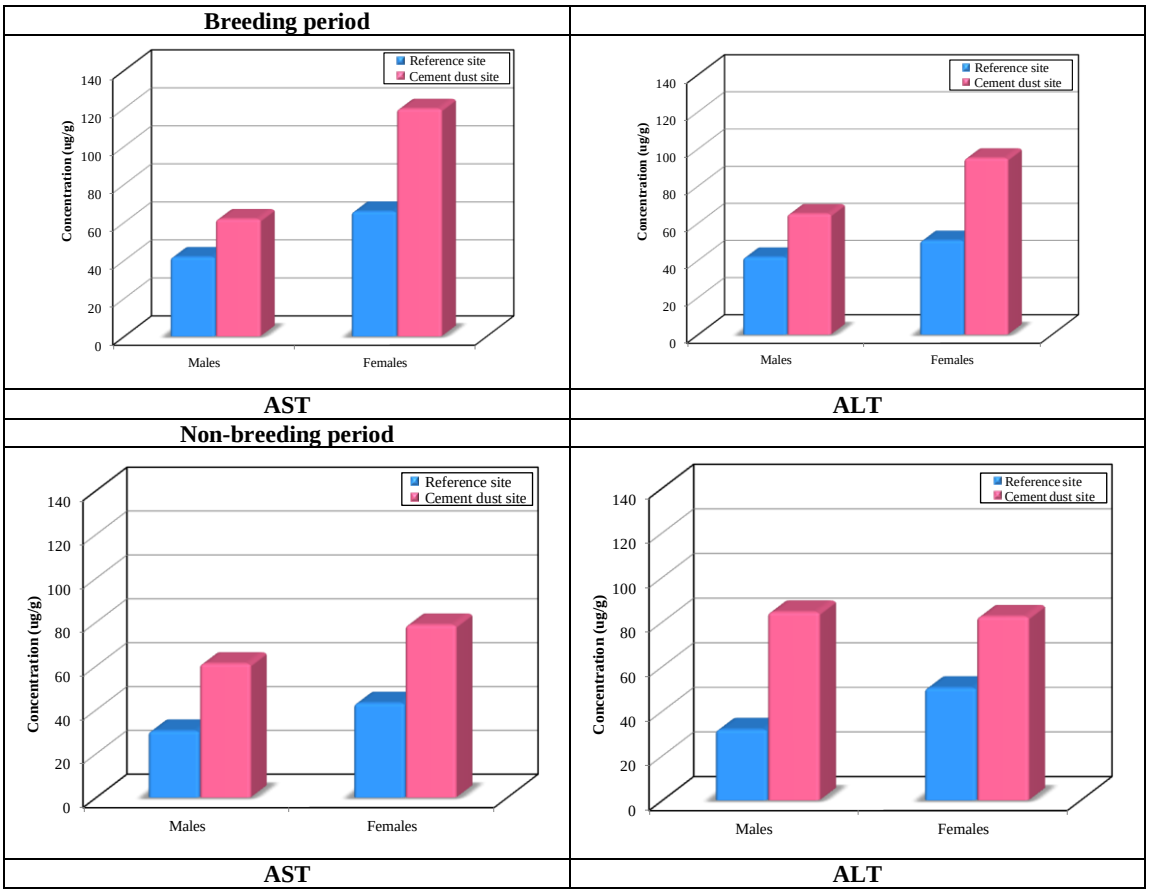


Fig 4: Levels of AST and ALT in males and females *T. hisipida* sampled from the inspected sites during breeding and non-breeding periods.

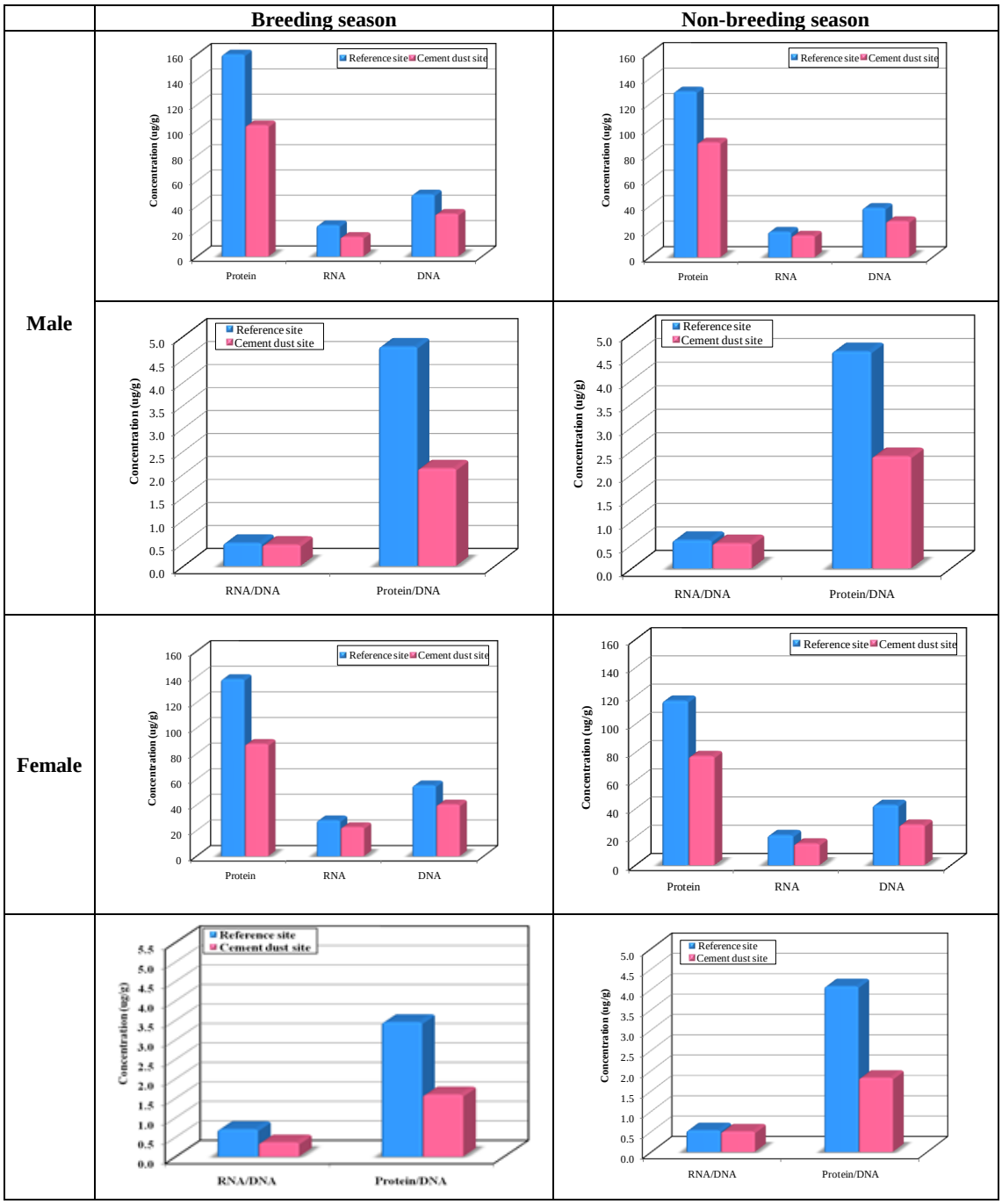


Fig 5: Protein and nucleic acid (RNA and DNA) content, RNA/DNA and Protein/DNA in body homogenate of male and female *T. hispida* sampled during breeding and non-breeding periods from both reference and cement dust sites.

4. Discussion

Environmental contaminants are one of the most challenging problems that face the world today. One of the most important reasons, which justify the need for a broad-scale, is cement dust pollution. There have been many reports on the occurrence of visible and subtle injuries induced by cement dust to human, plants and animals [31].

Cement dust contains several metals that enter the body of insect via the integument or through the intake of contaminated food and consequently they accumulated to a different extent in different organ/tissues. It was found in this study that the heavy metal concentrations in the soil were significantly higher in the cement dust site than reference site. These findings were in agreement with those obtained by Eckert *et al.* [32] and Poon & Chen [33] who demonstrated that cement dust and its products incorporated a high quantity of lead. Kulikov & Chelpanov [34] observed that cement dust application to soil increased concentrations of mobile trace elements. In the present study, leaf litter was found to contain significantly higher concentration of heavy metals in the cement dust than reference site. These data confirm those obtained by some previous studies such as Fakhry [35] who studied the effects of cement dust on plants present around the cement factory at El-Hammam area, and she found a decrease in both richness and diversity in the most affected site where only half the number of plant species could resist the disturbance of the cement factory. Cement dust is a potential source for many toxic pollutants and reducing hazardous effects on the plant growth [36].

AST and ALT are known to play a key role in the transamination processes in which a direct conversion of alpha-amino acid into the corresponding keto acids take place

as postulated early by Meister [37]. The present investigation revealed that the activity of AST as well as ALT was inhibited in both sexes of *T. hispida* collected during breeding and non-breeding periods as a result of increasing level of pollution by cement dust. The significant decrease in the activity of tissue transaminases of *T. hispida* collected from the polluted site suggested a functional damage in the cell led to the leakage of these cellular enzymes into the extracellular fluid. These findings are in agreement with those reported by many authors who suggested that there was a strong relationship between inhibition of tissue enzyme activities, specially transaminases, and tissue damage after treatment with different pesticides [38]. The observed decrease in transminases (AST, ALT) in entire body of both sexes of *T. hispida* may indicate the possible interference of pollutants with essential SH groups of enzymes. The inhibition exerted by metals on enzyme activity results from the interaction of a metal with enzyme SH groups [39]. Pollutant interacts with free-SH groups that are present in the activity in the active site of the enzyme and essential for enzyme activity as well as SH groups that are necessary for the stabilization of enzyme tertiary structure. Besides the reaction with -SH groups, blockage of –COOH groups with metals also appears to play a major role in inhibition of enzyme activity. It has been shown in various studies that toxic contaminants cause a change in protein content of the organisms studied [9, 40, 41]. Most studies that dealing with changes of protein levels in haemolymph and/or total body of insects, are restricted to Cd [42]. In Addition, Hussain and Jamil [43] found that accumulated Cd and Mg ions interfered with the protein synthesis whereas Zn demonstrated an opposite effect. In addition, Hussain and Jamil [44] stated that these heavy metals (Cd, Pb, and Zn) induced dramatic difference in protein profiles in the hyacinth weevil *Neochetina eichhorniae*. In addition, Ortel [45] found that feeding on Cd, Pb and Cd+Pb contaminated food, resulted in a significant decrease in protein, of pupae of *Ichneumonid pimpla turionella*. In vitro exposure to diet contaminated with Cd, Cu, Pb and Zn decreased total haemolymph protein in the insect, *Lymantria dispar* (Lepidoptera: Lymantriidae) [46]. A maximum reduction of protein content in insects feeding on Pb treated leaves (Jamil *et al*. [22] working on hyacinth weevil, *Neochetina eichhorniae*).

Data about the genome features in the present study showed that insects collected during breeding and non-breeding periods from the cement dust site displayed significant decrease in concentration of DNA, RNA, RNA/DNA ratio and protein/DNA (cell mass) as compared to those of reference site. It was previously documented that the amount of DNA is a measure of size for an individual [47] whereas the amount of RNA in cell is directly proportional to the protein synthesis rate. Therefore, the ratio of RNA/DNA is index of the metabolic rate of cells [48]. Enhanced level of RNA/DNA ratio was earlier shown as an indicator of growth competence of animals in healthy environment [28, 49]. The enhanced levels of this ratio in insect samples collected from the reference site proclaim that this site is more appropriate insect habitat. Some investigators have reported changes in DNA, RNA contents in different animal species as a result of biotranformation of xenobiotics (Jamil and Hussain, [22] working on the hycanith weevils *N. eichhorniae* and Dhawan, [50] working on *Drosophila melanogaster*). So, it could be concluded that *T. hispida* is severely affected by pollutants in the environment and the impact is obvious on the biochemical processes and cytogenetic parameters.

5. References

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