Archives of Environmental Sciences and Environmental Toxicology

OPEN ACCESS

El-Moaty ZA. Arch Environ Sci Environ Toxicol 6: 141.

www.doi.org/10.29011/2688-948X.100141

www.gavinpublishers.com

GAVIN PUBLISHERS

Research Article

Heavy Metals Concentrations and their Impact on the Enzyme Activities in Two Species of Darking Beetles; *Eleodes armata* (Le Conte) and *Eleodes tricostata* (Coleoptera: Tenebrionidae)

Zeinab A El-Moaty[1,2*]

[1]Department of Biological Sciences, college of Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia

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

[*]**Corresponding author:** Zeinab Abdel-Moaty Ahmed, Department of Biological Sciences, College of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia

Citation: El-Moaty ZA (2023) Heavy Metals Concentrations and their Impact on the Enzyme Activities in Two Species of Darking Beetles; *Eleodes armata* (Le Conte) and *Eleodes tricostata* (Coleoptera: Tenebrionidae). Arch Environ Sci Environ Toxicol 6: 141. DOI: 10.29011/2688-948X.100141

Received Date: 06 March 2023; **Accepted Date**: 15 March 2023; **Published Date**: 20 March 2023

Abstract

The contamination of environment with cement dust that contains heavy metals (HMs), is a severe problem due to adverse impact on the environmental safety and human health. This study has evaluated to quantify the concentration of heavy metals (Mg, Cu, Pb, Fe, Zn, Cd, and Ni), in the soil and accumulation of heavy metals in leaf litter as well as two species of beetles; *Eleodes armata* and *Eleodes tricostata* collected from surroundings of cement factory in Al-Ahsa, Eastern region of Saudi Arabia, comparing with reference site, that was away from the first one by about 30 Km. The results showed that for the cement site, the lowest concentration of HMs in soil samples was recorded for Cd (2.30±0.03 mg/kg), while the highest concentration was recorded for Pb (42.43±0.31 mg/kg). Concentrations of Cd in soil from the cement site was three times higher than in soil from reference site. Also, the concentration of HMs in soil, leaf litter and beetles collected from the two inspected sites were in order of Pb > Mg > Fe > Zn > Cu > Ni > Cd. Statistical analysis showed that the concentrations of HMs in soil, leaf litter and beetles collected from cement site were obviously significantly higher ($p<0.05$) than those recorded in different specimens collected from the reference site. The concentrations of Mg, Fe and Pb are the highest recorded concentrations of HMs for all collected samples. There were significant differences in their concentrations between the two selected species of beetles ($t = 5.37$, $p < 0.033$; $t = 11.31$, $p < 0.0001$; $t = 66.40$, $p < 0.0002$, respectively) collected from the cement site. Correlation coefficient showed that there was an accumulation of HMs from soil to leaf litter to beetles tissues. Moreover, the activities of transaminases; Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) as well as the protein content of both sexes of selected beetles' species were significantly lower in beetle tissues collected from the cement site than those collected the reference one.

Keywords: Ecotoxicology; Ground beetles; Heavy metals

Introduction

The cement industry is involved in the development of the physical infrastructure of advanced industrial society but generates dust during its production. Cement dust is considered as a major source of toxic air emission [1]. Cement dust contains toxic heavy metals, such as Cobalt (Co), Iron (Fe), lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Manganese (Mn), and arsenic (As) at different proportions. These heavy metals occupy low decay time and strong diffusivity, which in turn result in a considerable enrichment in soil and plants [2-4]. Uptake of heavy metals by two tobacco cultivars, caused a reduction on the growth and protein expression [5], alteration in morphological characters (such as chlorotic spot, stem shortening and leaf curling) and anatomical alterations (rupturing and thickening of cells) of *Cenchrus ciliaris* L. [6].

Accumulated HMs in soil transferred to invertebrates through the food chain via plants. This approach neglects possible differences in bioaccumulation between invertebrate species. Variability can be expected because of the variation in morphology, physiology, behavior, habitat and food preference between invertebrate species. Because of their lower metabolic rates and relatively simple enzymes system, insects may not be able to detoxify complex chemical compounds that they inhale or ingest with contaminated food. Residues of such persistent chemicals are laid down and transferred to a higher level in the food chain. Insects are usually used as bio-indicators of HMs, due to their mobility, abundance, and the variety of their physiology and morphology [7,8]. They can tolerate the accumulation of HMs in their tissues and organs, and they can survive in polluted soil because of their genetic adaptation [9,10].

Beetles are accumulators of HMs, particularly the remarkably toxic ones stored in gut [11]. Consequently, they are excellent indicators of habitat quality and are widely used in biological surveys. The selected species that belongs to Tenebrionidae; are scavengers feeding on decaying organic waste materials.

Eleodes beetles as essentially scavengers, feeding opportunistically as they wander through their habitat. *Eleodes armata* is one of the most conspicuous of tenebrionid beetles, not because it is particularly abundant, but because it is more frequently encountered during the daytime. The adult of *E. armata* is an elongate, shiny black, flightless beetle.

Because of the HMs accumulation, the biochemical stability had changed, causing physiological alterations in the beetles [12]. The detoxification (antioxidant) enzymes in insects are considered to be the defense mechanism against foreign components and have a role in preserve their normal physiological functions. Therefore, the assessment of the enzyme activities in insects exposed to HMs enables to evaluate the quantity of pollution. The exposure to heavy metals may cause stress reactions in insects: such as a reduction in antioxidant enzymes [13], an elevation in catalase (CAT) activity [14] or an increase in malondialdehyde (MDA) activity [15].

In this study, the HMs concentration in the soil, leaf litter and two species of darking beetle *E. armata* and *E. tricostata* collected from the region around the factory of cement, western region, KSA were evaluated and compared to those from the reference site. Moreover, the changes in activities of AST and ALT, as well as the protein content were evaluated in the selected two species of beetle tissues collected from the studied sites.

Materials and Methods

The study area

Two studied sites were selected; the first one was the cement site that surveyed at 0.5 km downwind from a cement factory in Al-Ahsa, about 30 Km to the south of Al- Ahsa city, in the Eastern region of Saudi Arabia (Figure 1). This area showed signs of cement dust deposition on the soil and surrounding vegetation. The second site is the reference site that was away from the first one by about 30 km.

Figure 1: Map of the studied region, Al-Ahsa city, Eastern region, Saudi Arabia.

Sampling procedure

The coleopterous insects inhabiting the selected sites were different species. Two species of beetles were selected; *E. armata* and *E. tricostata*. Soil, leaf litters and beetles' samples were collected from the two selected sites. From Each site, ten

samples of soil (each weighed about 0.5 kg, were collected from the top 10 cm by using a hand metallic soil scoop) and leaf litter were collected. Soil and leaf litter samples were transported to the laboratory in porous nylon bags. The collected beetles were separated, sorted by species and gender and maintained alive in containers in native soil and plants for 24 h to void gut contents. Beetles were kept under laboratory conditions (25±2°C, 60-70% relative humidity, and L14: D 10 photoperiod) until processing.

Chemical Analyses

For soil analysis, soil samples were air-dried at room temperature and ground to pass through a 0.2-mm sieve. Soil samples were placed in the digester for 8 h at 95°C with 10 ml of concentrated HNO_3. After soil digestion, samples were filtered and were made up to 100 ml by using distilled water. For leaf litter analysis, samples were dried at 70°C for two days and grounded. Samples were digested in concentrated sulfuric acid (H_2SO_4, 95-97%) on hotplate at approximately 270°C. Then, deionized water was added to 50 ml in a volumetric flask. 0.5 g of powdered samples of the leaf litter were carefully weighed in a 50 ml standard flask to which 5 ml of concentrated nitric acid was added. The mixture was left for 24 h and then the samples were digested on a hot plate and the temperature was gradually increased to 100°C. When all nitric oxide fumes were expelled, the temperature was raised and stabilized at 150°C until the mixture was clear. Distilled water was used to dilute the solution.

About 15 beetle samples were collected from each site and were anesthetized in ethanol (95%). After cutting the head, elytra, wings, and legs, the beetles dissected in Petri dishes containing drops of 1% Ringer's saline solution. The separated soft tissues were grounded and the prepared whole-body tissue samples subjected to digestion by adding 5-mL of supra-pure grade concentrated HNO_3 in the beaker and slightly heated up at 80°C to dehydration in the oven. After cooling, 5-mL of concentrated sulphuric acid (H_2SO_4) were added, and the mixture was heated for one hour then allowed to cool down. Then, 2-mL of 30% hydrogen peroxidase (H_2O_2) solution was added to the beaker contents and reheated. The final treatment reiterated until the mixture was clear [16].

The determination of HMs; Mg, Cu, Zn, Fe, Pb, Cd and Ni concentration of all collected samples were performed by using Atomic Absorption and Emission Spectrometry model Shimadzu-AA7000. Concentrations of heavy metals were determined according to Allen et al. [17]. All data are presented as concentration per unit wet weight of the sample (as mg/kg). All analyses were carried out on triplicate samples.

Biochemical analysis

Some biochemical parameters; enzyme levels of AST and ALT, and Protein content were determined in the soft tissues of males and females of the two selected beetle species, collected from the inspected sites. Levels of enzymes AST and ALT were assayed according to the method outlined by Nasr, et al. [15]. Protein concentration that assessed according to Crissman and Steinkamp [18], was measured in µg/100 mg tissue.

Statistical Analysis

Statistical analysis was performed using the program SPSS. Statistical analysis was carried out using an analysis of variance (ANOVA) according to Sokal and Rohlf [19] to analyze the significant differences in the HMs concentrations between the studied sites and the Pearson Correlation test to evaluate the relations between HMs concentrations in soil, leaf litter and beetles.

Results and Discussion

Bioaccumulation of heavy metals

Cement dust that originates from raw materials, contains HMs and deposited into soil. HMs are toxic to living organisms and could accumulated to a different extent in different organ/tissue [20]. In the cement site, the lowest concentration of HMs in soil samples was recorded for Cd (2.30±0.03 mg/kg), while the highest concentration was recorded for Pb (42.43±0.31 mg/kg) (Figure 2). This can be attributed by the fact that cement dust and its products incorporated a high quantity of lead [21]. Concentrations of Cd in soil from the cement site was three times higher than in soil from reference site. The concentration of HMs in soil collected from the two inspected sites were in order of Pb > Mg > Fe > Zn > Cu > Ni > Cd. Statistical analysis showed that the concentrations of Mg, Cu, Zn, Fe, Pb and Cd in soil collected from cement site were obviously significantly higher than those recorded in soil collected from the reference site. The concentration of Ni was insignificantly differed in soil collected from the studied sites (Table 1).

	One-way ANOVA	Heavy metals						
		Mg	Cu	Zn	Fe	Pb	Cd	Ni
Soil	F	14.64*	21.89*	49.21*	144.88*	723.19*	1999.39*	6.46
	P	0.01	0.01	0.002	0.0002	<0.0001	<0.0001	0.06
Leaf litter	F	16.31*	32.97*	12.224*	65.61*	284.69*	27.57*	1.235
	P	0.015	0.0045	0.025	0.0013	<0.0001	0.0063	0.323
*Statistically significant at $p \leq 0.05$								

Table 1: One-way analysis of variance of HMs concentrations in soil and leaf litters collected from the two studied sites.

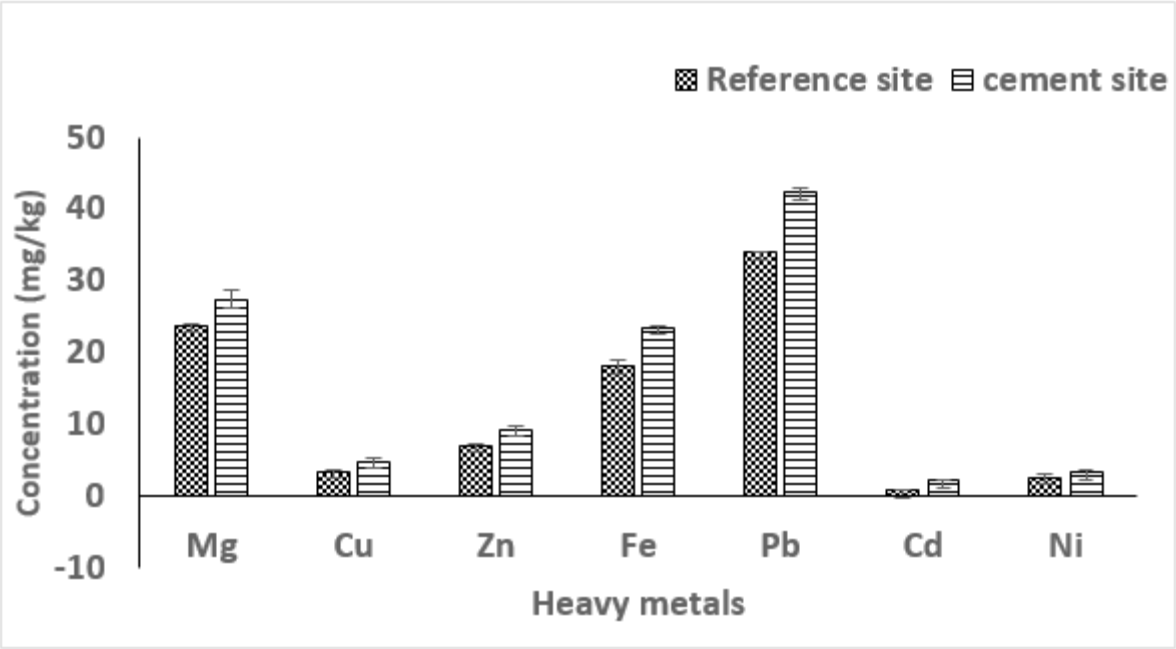

Figure 2: Heavy metals concentrations (mg/kg) in the soil collected from the two studied sites.

The concentrations of HMs in leaf litter have the same order recorded for soil, Pb > Mg > Fe > Zn > Cu > Ni > Cd (Figure 3). Based on the one way of ANOVA, there were significant differences between the concentrations of HMs in leaf litter collected from the reference site and those collected from the cement site (Table 1). Only, Ni concentration showed insignificant difference ($p > 0.05$) between the two selected sites. The HMs' concentrations in leaf litter were slightly higher than those recorded for soil. The concentrations of Mg, Fe and Pb are the highest concentrations of HMs, there were insignificant difference in their concentrations in soil and leaf litter ($t = 1.14$, $p > 0.30$; $t = 1.99$, $p > 0.20$; $t = 1.29$, $p > 0.30$, respectively).

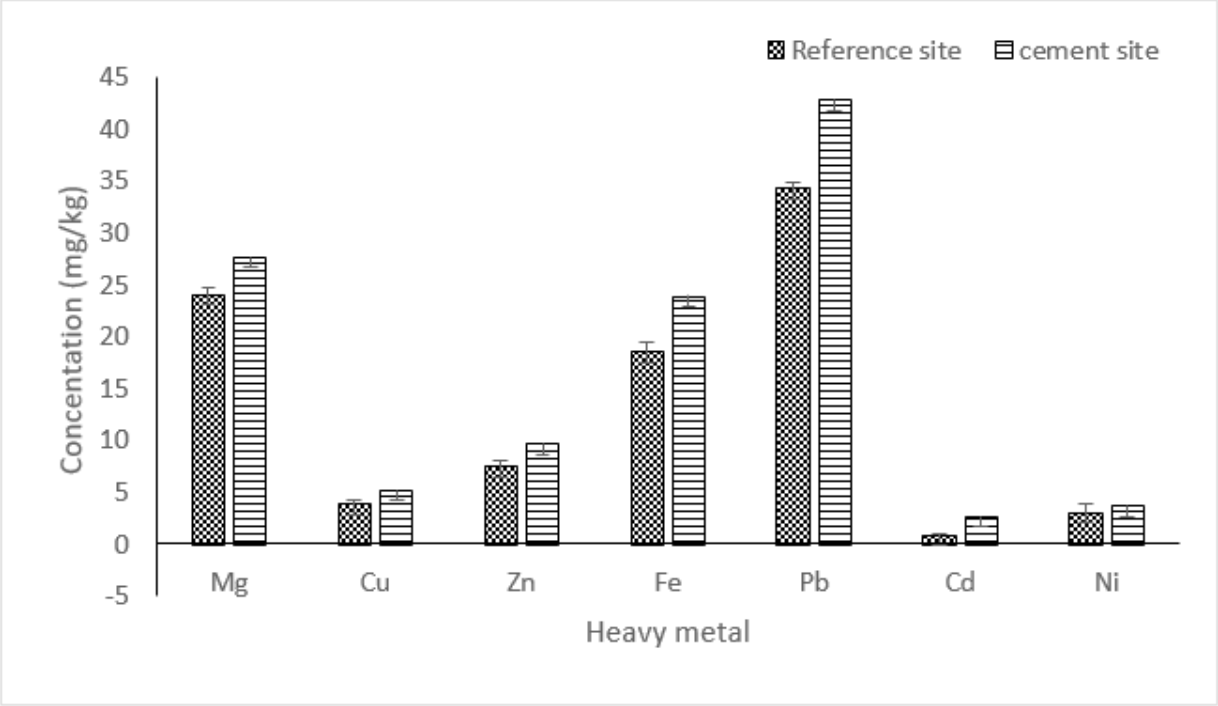

Figure 3: Heavy metals concentrations (mg/kg) in leaf litter collected from the two studied sites.

Cement dust reduces the absorption of mineral substances from the soil, resulting in changes in the plant physiology and morphology [22] as well as a reduction on the growth and protein expression [5]. In our study, the HMs' concentrations were significantly higher in leaf litter collected from the cement site than those collected from the reference site; these data confirm those obtained by some previous studies [6,23]. The present recorded concentration of Mg and Fe was higher in leaf litter collected from the cement site, these obtained results confirmed those obtained by Raajasubramanian et al. [24].

The recorded concentrations of HMs in *E. armata* and *E. tricostata* collected from the two studied sites were obviously showed that beetle accumulated HMs in the same order recorded for the soil and leaf litter (Pb > Mg > Fe > Zn > Cu > Ni > Cd) (Figure 4). However, studies on the leaf beetles, *Oulema gallaeciana* and *Lachnaia paradoxa* showed that the mean HMs levels in beetles decreased in the subsequent order Fe > Zn > Cu > Mg > Pb > Ni > Cd [23,25].

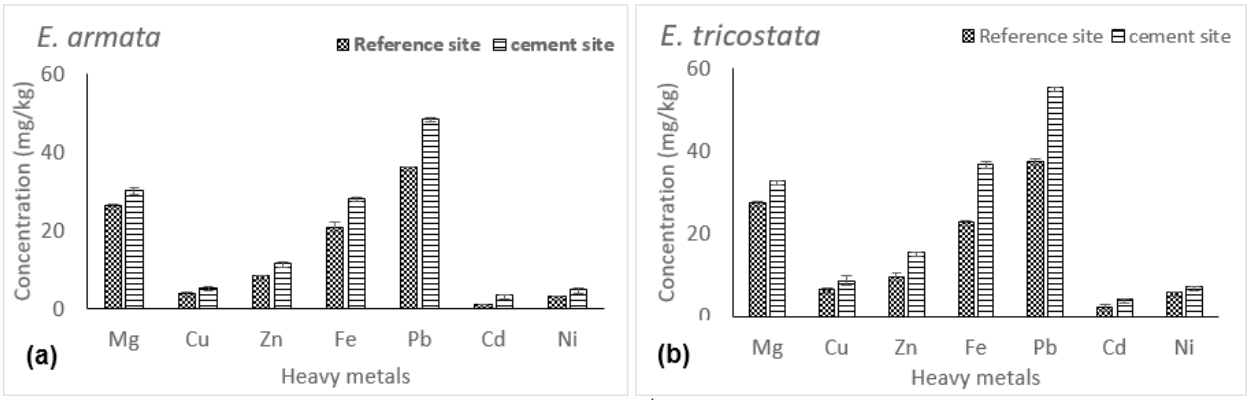

Figure 4: Heavy metals concentrations (mg/kg) in (a) *E. armata* and (b) *E. tricostata* collected from the two studied sites.

The statistical analysis showed that there were significant differences between the concentration of all HMs in *E. armata* and *E. tricostata* collected from cement site and those from the reference site (Table 2). These results concord with those results obtained by Walker et al. [26]; Naccarato, et al. [27] and Nasr et al. [15]. The concentrations of Mg, Fe and Pb are the highest concentrations of HMs, there were significant difference in their concentrations between the two selected species of beetles ($t = 5.37$, $p < 0.033$; $t = 11.31$, $p < 0.0001$; $t = 66.40$, $p < 0.0002$, respectively) collected from the cement site. The reported data showed different capabilities of the beetle species involved in accumulating HMs.

	One-way ANOVA	Heavy metals						
		Mg	Cu	Zn	Fe	Pb	Cd	Ni
E. armata	F	274.58*	26.62*	119.06*	84.54*	16001.75*	3182.65*	40.29*
	P	<0.0001	0.006	0.0004	0.0007	<0.0001	<0.0001	0.003
E. tricostata	F	141.25*	9.11*	76.21*	711.11*	2555.01*	29.11*	306.88*
	P	0.0002	0.04	0.001	<0.0001	<0.0001	0.005	<0.0001
*Statistically significant at $p \leq 0.05$								

Table 2: One-way analysis of variance of HMs concentrations in *E. armata* and *E. tricostata* collected from the two studied sites.

Additionally, the present results cleared that the two selected beetle species accumulated Cd and Ni less than Cu and Zn, these results similar to those recorded by Jelaska et al. [28] and Nasr et al. [11]. While, beetles accumulated high concentration of Pb, as recorded in Enchytraeus crypticus in highly contaminated habitats [29].

It is obvious that the concentrations of HMs were accumulated by transferring from soil to leaf litter and concentrated in beetle tissues (Figure 5). There was significant correlation of the concentration of HMs in soil, leaf litter and beetles (for Mg, Cu, Zn, Fe, Pb, Cd, and Ni the correlation coefficient was 0.963, 0.8471, 0.933, 0.9245, 0.949, 0.986 and 0.941, respectively). This biomagnification of metals has been reported at previous studies [28,30]. This increase of Pb and Cd concentration transferring from soil to plant to insect was observed in respect that Cd and Pb are non-essential elements [31]. It had reported that, metal associated with heat-stable, low molecular weight metal binding proteins, is highly available to predators, therefore, some complex mechanisms might exist in the higher tropic level of the food chain to tolerate the contaminated environment [29] (Table 3).

		Males			**Females**		
		AST	**ALT**	**protein content**	**AST**	**ALT**	**protein content**
E. armata	t	57	85	82	35.52	82	63.5
	p	<0.001	<0.003	<0.004	<0.0008	<0.0001	0.0002
E. tricostata	t	89	60.85	15.60	34.3	116	57.5
	p	<0.0001	0.0003	0.004	0.0008	0.001	0.003

Table 3: Statistical analysis of the activities of AST, ALT and protein content (µg/100 mg) in body of males and females (a) *E. armata* and (b) *E. tricostata* sampled from the inspected sites.

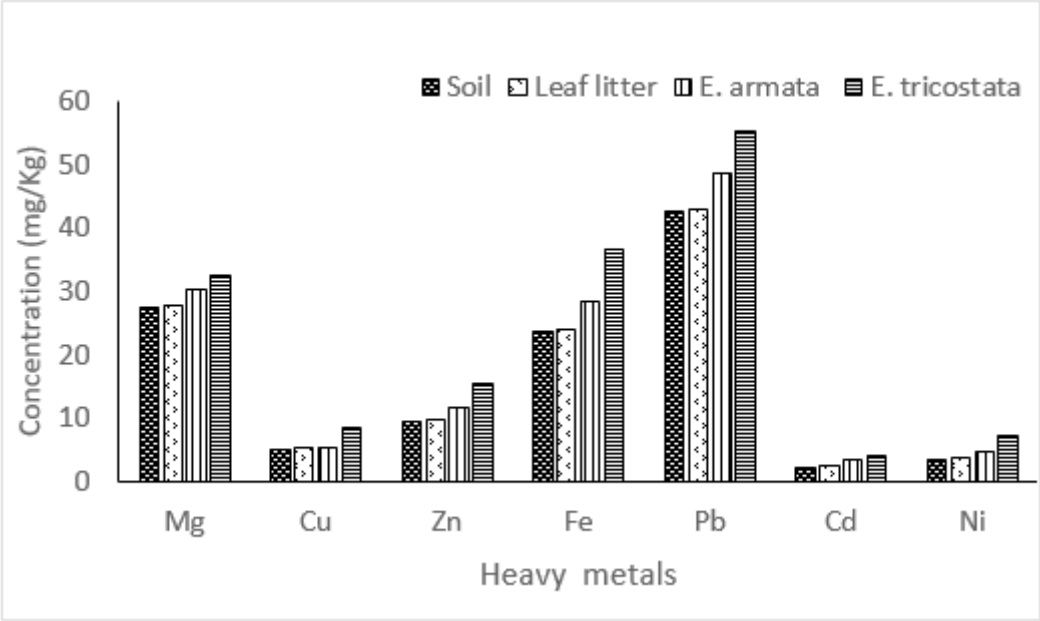

Figure 5: Heavy metal concentrations in soil, leaf litter, *E. armata* and *E. tricostata* collected from cement site.

Biochemical assessment

The levels of AST and ALT of males were lower than females of the two studied species of beetles collected from the two selected sites. While, protein content of males was higher than females of the two studied species of beetles collected from the cement site (Figure 6). The statistical analyses showed that the activities of transminases (AST, ALT) and protein content were decreased significantly ($p < 0.05$) in both sexes of the two studied species of beetles collected from the two selected sites. The accumulation of HMs in beetles inhibits the tissue enzyme activities, specially transaminases, and resulted in tissue damage. The reduction in enzyme activity could be explained due to the interaction of HMs with enzyme SH group. It has been shown in various studies that toxic contaminants cause a change in protein content of the organisms studied [32]. Ortel found that feeding on diet contaminated with Cd, Cu, Pb and Zn decreased total haemolymph protein in Lymantria dispar [33]. Also, a reduction in the CAT activity of male B. polychresta stressed with HMs could be due to decreased SOD activities at polluted sites [34].

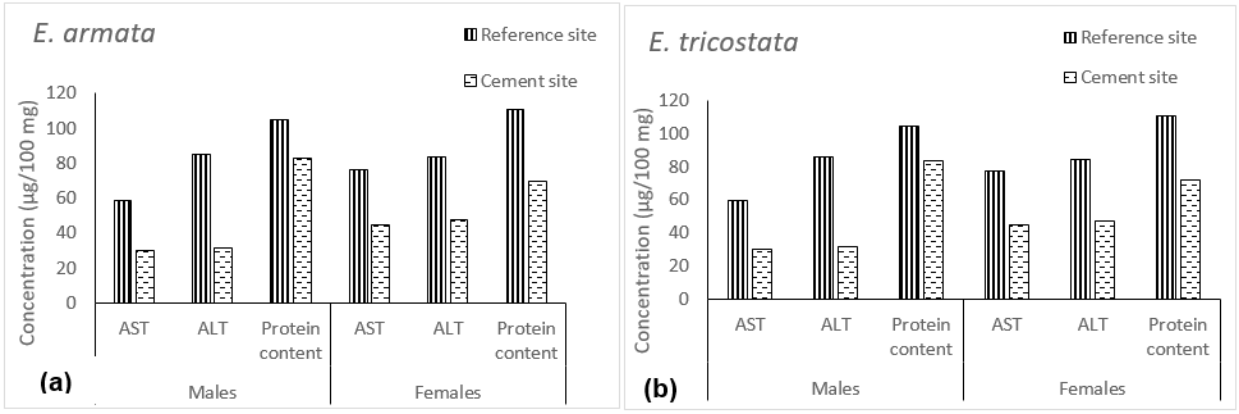

Figure 6: The activities of AST, ALT and protein content (µg/100 mg) in body of males and females (a) *E. armata* and (b) *E. tricostata* sampled from the inspected sites.

Conclusion

The heavy metals in the cement dust are present at different levels in the soil, plants and insects in the area around the industrial cement factory, Eastern region of Saudi Arabia. The data recorded in this study for HMs concentrations accumulated by transferring from soil to plant to insect. There was an accumulation of HMs from soil to leaf litter to beetles tissues. Moreover, the activities of transaminases; AST and ALT) as well as the protein content of both sexes were significantly lower in beetles' tissues collected from the cement site than the reference one.

Acknowledgements

The authors acknowledge the Deanship of Scientific Research at King Faisal University, Saudi Arabia, for the financial support under the annual research project (Grant No.2099).

References

1. Leem JH (2012) Cement dust and environmental diseases. Journal of the Korean Medical Association 55: 230-233.
2. Lamare RE, Singh OP (2020) Effect of cement dust on soil physico-chemical properties around cement plants in Jaintia Hills, Meghalaya. Environ Eng Res 25: 409-417.
3. Zhou X, Zhao C, Sun J, Cao Y, Fu L (2021) Classification of heavy metal Cd stress in lettuce leaves based on WPCA algorithm and fluorescence hyperspectral technology. Infrared Physics & Technology 119: 103936.
4. Sun W, Liu S, Zhang X, Li Y (2022) Estimation of soil organic matter content using selected spectral subset of hyperspectral data. Geoderma 409: 115653.
5. Shafiq M, Bakht J, Iqbal A, Shaft M (2020) Growth, protein expressionand heavy metal uptake by tobacco under heavy metals contaminated soil. Pakistan Journal of Botany 52: 1569-1576.
6. Al-faifi T, El-Shabasy A (2021) Effect of heavy metals in the cement dust pollution on morphological and anatomical characteristics of *Cenhrus ciliaris* L. Saudi Journal of Biological Science 28: 1069-1079.
7. El-Moaty ZA, Kheirallah DA, Elgendy DA (2016) Impact of Cement Dust on Some Biological Parameters of *Trachyderma hispida* (Coleoptera: Tenebrionidae) Inhabiting the vicinity of a cement factory, Mariout region, Alexandria, Egypt. Journal of Entomology and Zoology Studties 4: 797-805.
8. Singh S, Diksha D, Mahajan E, Sohal SK (2022) Chapter 15 - Effect of heavy metals on insects. Appraisal of Metal(loids) in the Ecosystem. pp: 361-390.
9. Ghemari C, Ayari A, Hamdi N, Waterlot C, Douay F, et al. (2018) Measure of environmental stress on *Porcellio laevis* Latreille, 1804 sampled near active Tunisian industrial areas. Ecotoxicology 27: 729-741.
10. Dar MI, Green ID, Khan FA (2019) Trace metal contamination: transfer and fate in food chains of terrestrial invertebrates. Food Webs 20: e00116.
11. Nasr EE, Khater ZZ, Zelenakova M, Vranayova Z, Abu-Hashim M (2020) Soil physicochemical properties, metal deposition, and ultrastructural midgut changes in ground beetles, *Calosoma chlorostictum*, under agricultural pollution. Sustainability 12: 4805.
12. Bednarska AJ, Stachowicz I (2013) Costs of living in metal polluted areas: respiration rate of the ground beetle *Pterostichus oblongopunctatus* from two gradients of metal pollution. Ecotoxicology 22: 118-124.
13. Stone D, Jepson P, Laskowski R (2002) Trends in detoxification enzymes and heavy metal accumulation in ground beetles (Coleoptera, Carabidae) inhabiting a gradient of pollution. Comp Biochem Physiol C Toxicol Pharmacol 132: 105-112.
14. Laszczyca P, Augustyniak M, Wilczek G, Rozp E, et al. (2004) Antioxidative defense enzymes in beetles from a metal pollution gradient. Biologia (Bratislava) 59: 645-654.
15. Nasr EE, Algefare AI, Al-Gabri NA, Khater ZZ (2021) Enzymatic disturbances of the Egyptian beetle, *Blaps polychresta* (Forskål, 1775), as environmental indicators of heavy metal pollution of agricultural soils. Annual of Agriculture Science 66: 63-74.
16. Talarico F, Brandmayr P, Giulianini PG, Ietto F, Naccarato A, et al. (2014) Effects of metal pollution on survival and physiological responses in Carabus (Chaetocarabus) lefebvrei (Coleoptera, Carabidae). European Journal Soil Biology 61: 80-89.
17. Allen S, Grimshaw H, Rowland A (1986) Chemical Analysis. Methods in Plant Ecology Back Well Scientific Publications.
18. Crissman HA, Steinkamp JA (1994) Chapter 11 Cellular Protein Content Measurements. Methods in Cell Biology 41: 175-183.
19. Sokal RR, Rohlf FJ (2012) Biometry: The Principles and Practice of Statistics in Biological Research. 4th Edition. W.H. Freeman and Company, New York.
20. Schuhmacher M, Nadal M, Domingo JL (2009) Environmental monitoring of PCDD/Fs and metals in the vicinity of a cement plant after using sewage sludge as a secondary fuel. Chemosphere 74: 1502-1508.
21. Silva TC, Paula MJ, Silva WS, Lacprte GA (2021) Deposition of potentially toxic metals in the soil from surrounding cement plants in a Karst area of southeastern Brazil. Conservation 1: 137-150.
22. Zafar IM, Shafiq M (1998) Toxicity of cement dust on the growth of some tree seedlings. Ekologia 17: 434-439.
23. Butovsky RO (2011) Heavy metals in carabids (Coleoptera: Carabidae). Zookeys 100: 215-222.
24. Raajasubramanian D, Sundaramoorthy P, Baskaran L, Sankar Ganesh K, Chidambaram A, et al. (2011) Cement dust pollution on growth and yield attributes of groundnut (*Arachis hypogaea* L.). International Multidisciplinary Researches Journal 1: 31-36.
25. Ghannem S, Touaylia S, Bejaoui M (2018) Assessment of trace metals contamination in soil, leaf litter and leaf beetles (Coleoptera, Chrysomelidae) in the vicinity of a metallurgical factory near Menzel Bourguiba (Tunisia). Human Ecological Risk Assessment 24: 991-1002.
26. Walker CH, Sibly RM, Hopkin SP, Peakall DB (2012) Principles of Ecotoxicology. 4th Edition. Taylor and Francis, Boca Raton, Florida, USA.
27. Naccarato A, Tassone A, Cavaliere F, Elliani R, Pirrone N, et al. (2020) Agrochemical treatments as a source of heavy metals and rare earth elements in agricultural soils and bioaccumulation in ground beetles. Sci Total Environ 749: 141438.
28. Jelaska LS, Blanusa M, Durbesic P, Jelaska SD (2007) Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem. Ecotoxicology Environmental Safety 66: 74-81.
29. Zhang LC, Van Gestel AM (2017) Toxicokinetics and toxicodynamics of lead in the soil invertebrate *Enchytraeus crypticus*. Environ Pollut 225: 534-541.

30. Goodyear KL, McNeill S (1999). Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. Sci Total Environment 229: 1-19.

31. Heikens A, Peijnenburg WJGM, Hendriksa AJ (2001) Bioaccumulation of heavy metals in terrestrial invertebrates. Environ Pollut 113: 385-393.

32. Oruç EÖ, Usta D (2007) Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. Environm Toxicol Pharmacol 23: 48-55.

33. Ortel J (1995) Changes in protein content and free amino-acid composition in metalo-contaminated gypsy moth larvae (*Lymantria dispar* L., Lymantriidae, Lepid.). Comp Biochem Physiol - Part C: Toxicol and Pharm 112: 291-298.

34. Kolawole AO, Olajuyigbe FM, Ajele JO, Adedire CO (2014) Activity of the antioxidant defense system in a typical bioinsecticide-and synthetic insecticide-treated cowpea storage beetle *Callosobrochus maculatus* F. (Coleoptera: Chrysomelidae). Int J Insect Sci 6: 99-108.