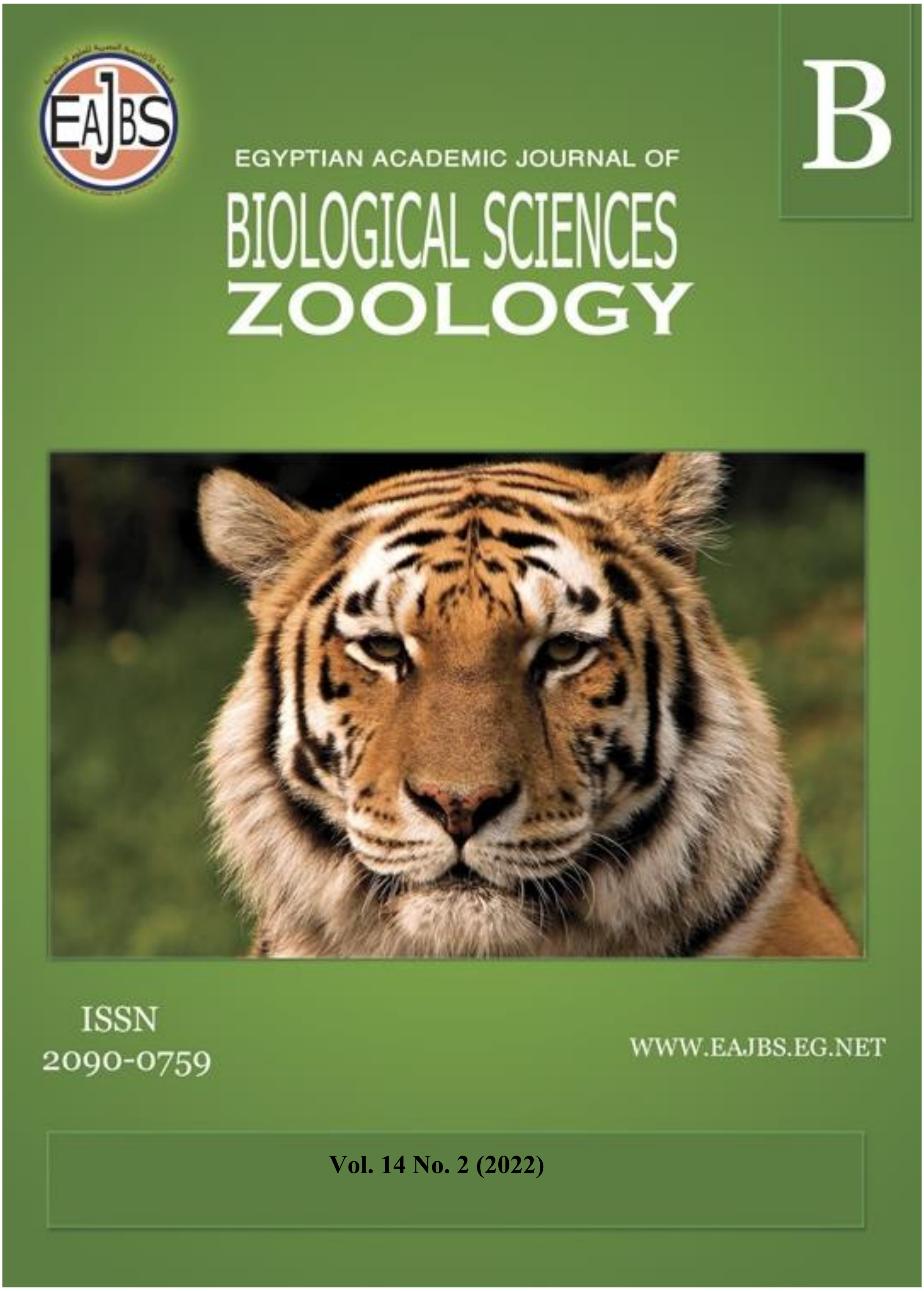

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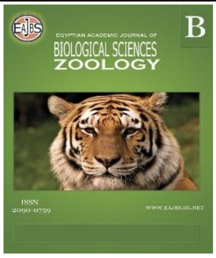

Bioindication of Beetles and Spiders for the Ecotoxicological Study of the Impact of Heavy Metals and their Interaction with Detoxification Enzymes

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ABSTRACT

The utilization of beetles and spiders has recently become a great mean for a fast and accurate trend in the ecotoxicological study. Tenebrionid beetle (*Mesostena angustata*) and carnivorous spider (*Galeodes arabs*) were chosen as bioindicators to evaluate the industrial impact in El Sadat industrial region, Menofia, Egypt. Three categories of industries; light, medium, and heavy industries compared with the natural environment were selected. Bioaccumulation of heavy metal body burden and the interacted activity of detoxification enzymes: Acetylcholinesterase and Lactate Dehydrogenase were assessed in both species during four successive seasons. Results showed significant variations in the seasonal and spatial bioaccumulation of heavy metals in tested species. Summer was the highest season with heavy metal accumulation amongst the heavy industries, followed by the medium industries. That was confirmed later with the Metal pollution index. *M. angustata* was more susceptible to heavy metal accumulation than *G. arabs.* The major accumulated elements were; iron and zinc opposite to cadmium in both species. Consequently, the increased activity of both detoxifying enzymes revealed they are related to the bioaccumulation of heavy metals, particularly in the zone of heavy industries during the summer season. For confirmation, the significant positive relationship was between total accumulated heavy metals and detoxification enzyme activity.

INTRODUCTION

Tracing the impact of industrial pollution is one of the most difficult tasks due to its considerable accumulation impact and elevated persistence. Continuous accumulation of heavy metals had a dangerous anthropogenic impact on the surrounding ecosystem due to their long-lasting characteristics in soil or their translocation via food webs or food chains which directly affect the sustainability of ecological systems (Wuana and Okieimen, 2011; Opaluwa *et al*., 2012).

Bioaccumulation of heavy metals varies according to differences in the physiology, behaviour and trophic levels between different groups of organisms (Hendrickx *et al*., 2003; Wilczek *et al*., 2008). Insects are considered representative ecotoxicological tools due to their link in metal accumulation and the transportation chain between the trophic levels which clarifies heavy metals' distribution in the biosphere as

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well as the index of metal pollution (Lindqvist and Block, 1995). Among the insects, the coleopteran family (Tenebrionidae) are considered good bioindicators for soil pollution due to their habitat variation that resembles almost all other biogeographical regions in addition to the anthropogenically-induced landscape modification (Osman *et al.*, 2015). Among the carnivorous invertebrates; spiders are known to accumulate higher concentrations of heavy metals, so they are regarded as macro-concentrators (Hendrickx *et al*., 2003).

Various mechanisms deal with additional heavy metals accumulation stress as storage of metals in different organs of their body or the elimination from their bodies by excretion or molting (Lee *et al*., 1978; Ludwig and Alberti, 1988). Besides, variation in detoxification enzyme activity was responded to different xenobiotics and heavy metals by metabolism, degradation, or even antioxidative activity to protect vital body organs or molecules (Wilczek *et al*., 2008). Therefore, the activity of detoxification enzymes has been widely used as an early sensitive, responsive tool and efficient biochemical biomarker in the assessment of environmental contamination (Van Praet *et al.*, 2014; Bream *et al*., 2019).

In this study, tracing the impact of industrial pollution on the surrounding environment by detecting bioaccumulation of heavy metals throughout the trophic food chain in tenebrionid beetle and carnivorous spider and estimation detoxification enzymes activity as a biomarker for industrial impact and the relationship between bioaccumulation of heavy metals and the activity of detoxification enzymes were the main aims of this study.

MATERIALS AND METHODS

Study Area:

El Sadat industrial region represents one of the largest industrial masses in the Egyptian Delta, Menofia Governorate. It is famously served with its locality at central Delta between both Nile River branches; Damietta (Dumyat) and Rosetta (Rashid) branches, and its nearness to the Cairo-Alexandria highway and its surrounding green belt earned it a place in the top ten lists of environmental industrial cities in the Middle East (Ayyad 2016).

It attracted investments in different fields of industries starting from small workshops to heavy industries companies. Three main groups of industries were classified and they include viz; light industries- such as food and drinking industry and different companies for import and export; medium industries- such as weaving, spinning and dyeing companies; and heavy industries- such as iron and steel, engineering and metal products, electronic and electric appliances, wood and furniture products, plastics, chemicals, vehicles, industrial gases, textile, paper products and medical products companies. These study zones were chosen to represent the impact of different levels of industries in this region throughout the bioaccumulation of heavy metals. Each zone clarified well the nature of impact within a wide area and the coordinates of each one were recorded using a hand-held Global Positioning System (Fig. 1).

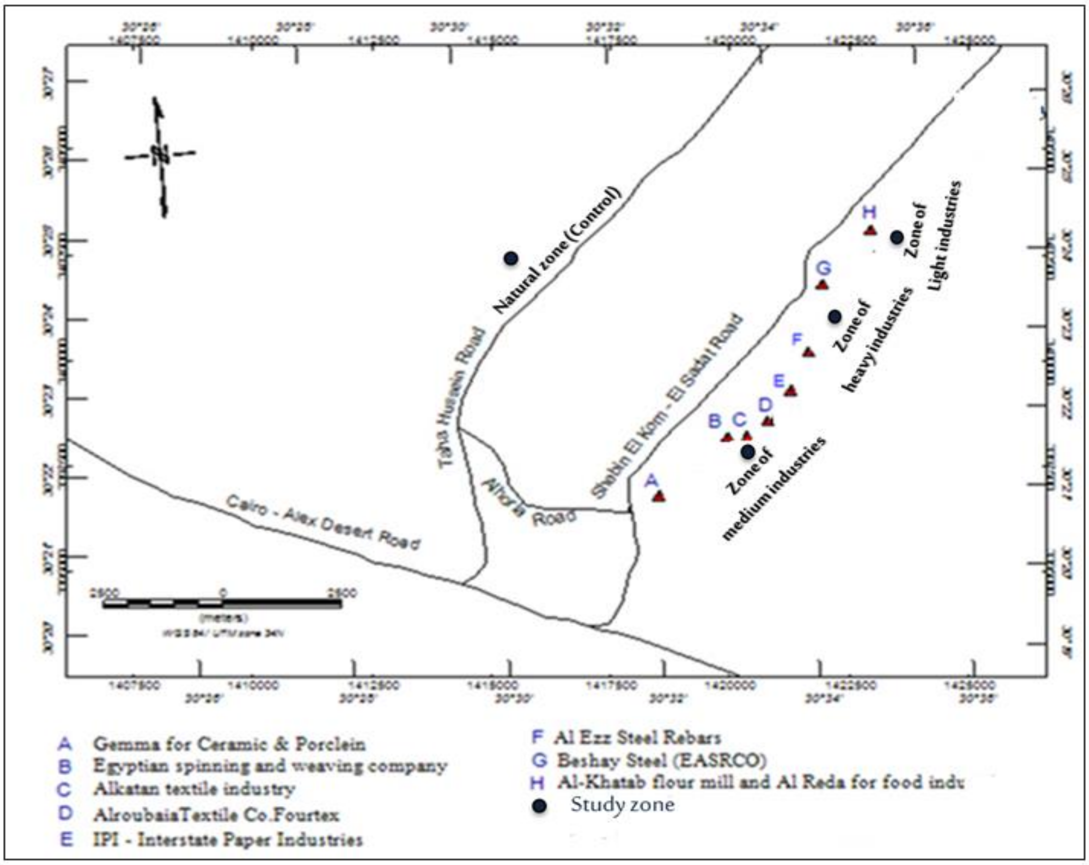

Fig.1. Map of the current study area, natural zone (control) and different industrial zones attributing to different factories in El Sadat industrial region in Menofia Governorate, Egypt.

Detection of Heavy Metals Accumulation:

For both tested species, the whole insect was weighed, labelled according to sampling site and season and stored at - 4˚C for 24 hours. Pre-digestion of these samples was begun with analar nitric acid (0.5 - 1 ml/ 25 mg tissue) then gently heated in the heating block (90°C) and vortexed to help in tissue solubilization. Dissolved samples were returned to the heating block till the color starts to turn brown. Then, cooling was carried out and 0.1 ml of concentrated nitric acid was added. Reheating till the volume of solution was reduced to 0.25 - 0.5 ml was carried out. Cooling of samples was carried out again and 0.1 ml of peroxide H_2O_2 (30%) was added. Reheating of the dissolved samples was done in the heating block. Digested tissues were diluted with distilled water to 2 ml. A blank solution was prepared to check any possible trace metals that may be present in the acid or distilled water used in digestion. Samples were analyzed using GBC atomic absorption spectrophotometer (GBC, Savanta AA) in the National Institute of Ocean and Fish, Egypt according to Sachdev and West (1970). Results were expressed in ppm dry weight of the tissue homogenate.

Enzymatic Activity Assay:

The current study pointed to the use of specific enzymes; AChE and LDH for monitoring industrial pollution as useful biomarkers. Samples preparations were carried out using the whole-body tissue homogenate. Homogenization was done in saline solution by the following rate (1 g tissue: 1 ml saline solution 0.7%) using a fine polytron homogenizer for 2 mins. Then, homogenate was ice-centrifuged at 4000 r.p.m/ 15 min. The supernatant was directly assayed or frozen until measurement. Kits for AChE concentrations were from (Quimica Clinica Aplicada S.A. Company, Egypt). While, LDH kites were purchased from Biosystem reagents, according to the technique adopted by Ellman *et al.* (1961). Enzyme activity was detected at wavelength 340 nm by spectrophotometer according to the method of Tietz (1999). Three replicates were prepared for each measurement.

Statistical Analysis*:*

Data entry was done using Excel 365. Descriptive statistics including mean and standard error (SE) was calculated for each category. One-Way Analysis of Variance (ANOVA) and Pearson correlation coefficient was done using SPSS ver. 25 for Windows 7. Holm Sidak post hoc method was used for pairwise comparisons. Figures were graphed by Sigma Plot version 11.0. Data presented as Mean ± Standard Error (SE). *P*-value was considered significant at < 0.05.

RESULTS

Bioaccumulation of Heavy Metals:

Variation in seasonal and spatial bioaccumulation of heavy metals is given in (Table 1). Seasonally, there was an extremely significant difference in bioaccumulation of heavy metals levels among the different industrial zones investigated for almost all the investigated heavy metals except cadmium which showed very low levels during the summer season in beetle species, *M. angustata* and winter season in spider species, *G. arabs*.

Overall Pattern of Heavy Metals Bioaccumulation in Tenebrionid Beetle:

For seasonal and spatial bioaccumulation of heavy metals of *M. angustata*, cadmium was the metal with the lowest accumulation levels across the different investigated seasons (Table 1). Its values ranged between 0.8 ± 0.05 ppm in the natural environment during the spring season and 4.7 ± 0.3 ppm in a zone of heavy industries during the autumn season. Iron recorded the highest accumulated concentrations, particularly in the heavy industries region followed by zinc. The summer season particularly attained the highest levels of iron accumulation. The highest recorded accumulation level of iron (502.1 ± 0.6 ppm) was in the summer season in the heavy industries region followed by 422.2 ± 1.5 ppm in the medium industries region during the same season. For zinc, the highest accumulation levels were recorded in the heavy industries region with 156.0 ± 0.6 ppm and 118.2 ± 4.6 ppm recorded in the summer and winter seasons, respectively. Surprisingly, manganese was not detected in the natural environment during autumn with very low detected concentrations in other investigated regions.

Overall Pattern of Heavy Metals Bioaccumulation in Spider:

Regarding heavy metals bioaccumulation in *G. arabs* (Table 1), cadmium was the lowest accumulated metal in different investigated seasons followed by and copper and manganese. In the heavy industries region, the highest level of manganese accumulation (26.5 ± 0.5 ppm) was recorded in the winter season, followed by copper (18.5 ± 0.28 ppm) in the same season. The winter season attained the highest levels of iron and zinc accumulation. The highest accumulation level recorded was for Zinc (126.5 ± 0.3 ppm) followed by iron (111.0 ± 0.12 ppm) in the heavy industries region. By region, accumulation levels of iron and zinc may be arranged increasingly as the following: Light< Medium < heavy. Interestingly, manganese was detected only in the heavy industries region with a very low concentration (0.3 ± 0.01 ppm) during the spring season.

By comparing both tested species, accumulates higher levels of iron, zinc, copper and manganese during the summer season. Meanwhile, *G. arabs*, accumulated high levels of iron during the summer season in almost all tested regions. While, the different metals: zinc, manganese, and copper were highly accumulated during the winter season (Table 1).

Table 1: Spatial and seasonal variation in bioaccumulation of heavy metals (ppm) in *Mesostena angustata (*Coleoptera: Tenebrionidae) and spider species, *Galeodes arabs*, as bioindicators for different levels of industries zones in El Sadat industrial region, El Sadat city, Menofia Governorate, Egypt.

Metal	Zone	Heavy metals concentrations (ppm) in *Mesostena angustata*				Heavy metals concentrations (ppm) in *Galeodes arabs*			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Cadmium (Cd)	Cont.	0.8 ± 0.05^{b}	2.3 ± 0.3^{b}	2.2 ± 0.3^{c}	1.8 ± 0.3^{ab}	0.86 ± 0.06^{b}	1.7 ± 0.4^{b}	4.3 ± 0.2^{b}	1.7 ± 0.57^{a}
	Light indust.	1.2 ± 0.05^{b}	2.6 ± 0.3^{b}	2.7 ± 0.3^{bc}	1.9 ± 0.3^{ab}	0.87 ± 0.11^{b}	2.2 ± 0.12^{b}	4.8 ± 0.11^{ab}	1.8 ± 0.05^{a}
	Medium indust.	1.5 ± 0.1^{b}	3.4 ± 0.3^{a}	2.8 ± 0.3^{b}	2.0 ± 0.3^{b}	1.4 ± 0.11^{a}	2.4 ± 0.23^{ab}	5 ± 0.6^{ab}	2.0 ± 0.05^{a}
	Heavy indust.	2.5 ± 0.29^{a}	3.8 ± 0.1^{a}	4.7 ± 0.3^{a}	2.7 ± 0.3^{a}	2.4 ± 0.2^{a}	3.5 ± 0.3^{a}	6.1 ± 0.05^{a}	2.2 ± 0.57^{a}
Copper (Cu)	Cont.	0.2 ± 0.05^{b}	10.5 ± 0.23^{c}	2.1 ± 0.4^{c}	6.5 ± 0.4^{c}	0.13 ± 0.08^{c}	0.01 ± 0.001^{c}	0.2 ± 0.05^{c}	3.8 ± 0.133^{c}
	Light indust.	0.27 ± 0.07^{b}	12.4 ± 0.1^{b}	2.8 ± 0.4^{bc}	6.8 ± 0.4^{c}	0.2 ± 0.05^{c}	1.2 ± 0.11^{c}	4.1 ± 0.11^{b}	5.0 ± 0.6^{b}
	Medium indust.	$0.4 \pm 0.0.1^{b}$	13.466 ± 0.6^{b}	4.3 ± 0.4^{ab}	10.7 ± 0.4^{b}	2.3 ± 0.17^{b}	3.0 ± 0.7^{b}	5.2 ± 0.3^{b}	7.6 ± 0.23^{b}
	Heavy indust.	2.8 ± 0.37^{a}	39.76 ± 0.1^{a}	4.7 ± 0.4^{a}	18.5 ± 0.4^{a}	4.0 ± 0.5^{a}	6.7 ± 0.11^{a}	7.5 ± 0.3^{a}	18.5 ± 0.28^{a}
Iron (Fe)	Cont.	62.8 ± 0.46^{b}	218.2 ± 0.6^{b}	15.6 ± 0.2^{c}	6.03 ± 0.3^{d}	33.2 ± 0.11^{c}	100 ± 1.8^{b}	23.0 ± 0.17^{c}	60.2 ± 0.11^{c}
	Light indust.	66.7 ± 3.5^{b}	218.97 ± 0.6^{c}	32.45 ± 0.3^{b}	25.6 ± 1.4^{c}	35.5 ± 0.12^{c}	102 ± 0.3^{b}	37.07 ± 0.26^{d}	68.4 ± 0.138^{b}
	Medium indust.	79.3 ± 4.1^{a}	422.2 ± 1.5^{b}	33.5 ± 1.88^{b}	151.4 ± 0.4^{b}	55.5 ± 0.57^{b}	102.2 ± 1.3^{b}	39.0 ± 0.5^{a}	97.7 ± 0.13^{a}
	Heavy indust.	87.2 ± 0.57^{b}	502.1 ± 0.6^{a}	223.3 ± 0.17^{a}	274.2 ± 2.3^{a}	90.4 ± 0.24^{a}	120.1 ± 1.1^{a}	49.1 ± 0.05^{a}	111.0 ± 0.12^{b}
Manganese (Mn)	Cont.	0.03 ± 0.03^{d}	7.466 ± 0.3^{c}	--	2.7 ± 0.1^{c}	--	4.3 ± 0.17^{d}	4.2 ± 0.2^{b}	8.8 ± 1.2^{b}
	Light indust.	3.3 ± 0.2^{c}	17.5 ± 0.14^{b}	0.2 ± 0.05^{a}	5.8 ± 0.46^{b}	--	7.6 ± 0.33^{c}	4.2 ± 0.11^{b}	11.2 ± 1.2^{b}
	Medium indust.	4.9 ± 0.5^{b}	20.4 ± 0.5^{b}	0.2 ± 0.05^{a}	5.5 ± 0.288^{b}	--	11.8 ± 0.2^{b}	6.3 ± 0.57^{a}	25.2 ± 0.12^{a}
	Heavy indust.	9.4 ± 0.2^{a}	27.4 ± 1.8^{a}	0.3 ± 0.05^{a}	13.8 ± 0.46^{a}	0.3 ± 0.01	18.7 ± 0.2^{a}	7.0 ± 0.22^{a}	26.5 ± 0.5^{a}
Zinc (Zn)	Cont.	55.1 ± 2.9^{b}	121.1 ± 1.78^{c}	23.1 ± 1.7^{c}	57.8 ± 0.46^{b}	23.5 ± 0.3^{d}	58.9 ± 0.6^{c}	33.7 ± 1.2^{d}	38.6 ± 0.57^{b}
	Light indust.	58.3 ± 0.2^{b}	59.433 ± 0.2^{d}	25.5 ± 0.3^{c}	68.6 ± 0.6^{b}	32.5 ± 1.4^{c}	63.8 ± 0.5^{d}	45.5 ± 0.001^{c}	105 ± 1.2^{b}
	Medium indust.	62.1 ± 1.2^{b}	130.6 ± 0.7^{b}	31.3 ± 0.6^{b}	66 ± 1.55^{b}	45.5 ± 0.8^{b}	98.8 ± 2.9^{a}	51.4 ± 0.23^{b}	107 ± 1.5^{b}
	Heavy indust.	96.6 ± 1.78^{a}	156.0 ± 0.6^{a}	37.2 ± 0.05^{a}	118.2 ± 4.6^{a}	72.3 ± 0.17^{a}	98.4 ± 0.2^{b}	57.4 ± 0.23^{a}	126.5 ± 0.3^{a}

Data were analyzed by one-way ANOVA, followed by Holm Sidak post hoc test and presented as Mean ± SE of three replicates. For each determined metal, a column with means followed by different letters differs significantly, $P < 0.05$. (Cont.): zone of the natural environment at control, (Light indust.) zone of light industries, (Medium indust.) zone of medium industries, and (Heavy indust.) zone of heavy industries.

Bi-annual accumulation of heavy metals was given in (Fig. 2), where summer followed by winter seasons attained higher levels of heavy metals accumulation in comparison with spring and autumn seasons for *M. angustata* and *G. arabs*. Moreover, *M. angustata* was more susceptible to heavy metals bioaccumulation than *G. arabs*.

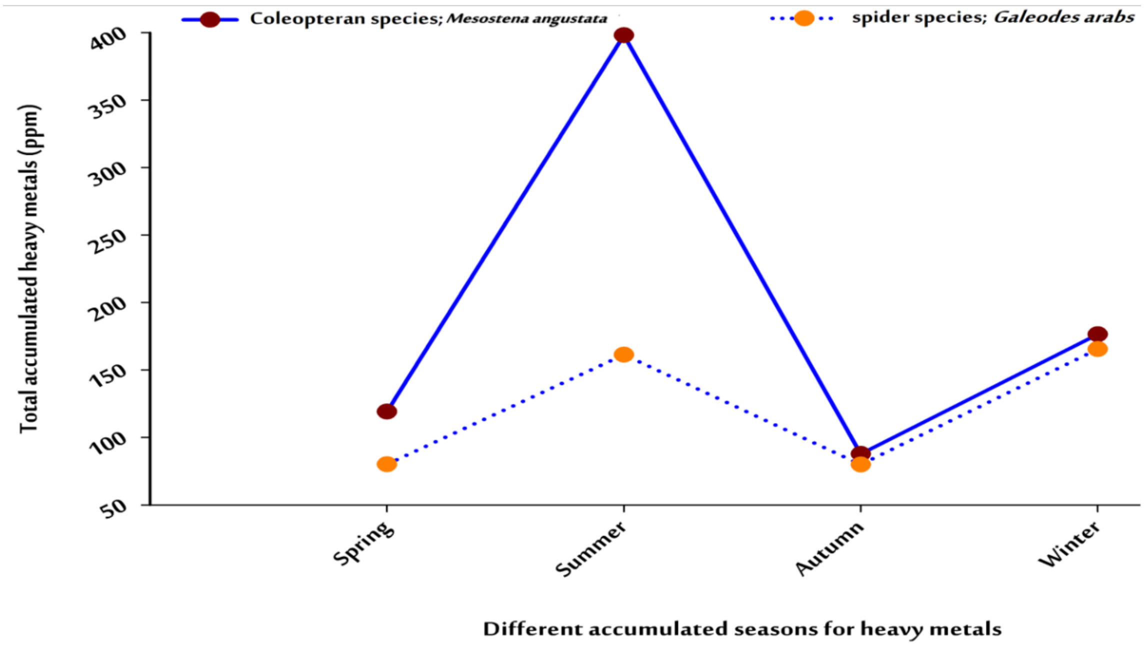

Fig.2: Seasonal variation in the average amount of heavy metals bioaccumulation for different species sampled in El Sadat industrial region, El Sadat city, Menofia Governorate, Egypt.

Spatial Variation in Species Bioaccumulation:

By comparing heavy metals bioaccumulation in coleopteran species, *M. angustata* we found that iron > zinc were the major accumulated metals in this species (Fig. 3). On the other hand, the spider species, *G. arabs* exhibited the same accumulation pattern with almost the same concentrations for iron and zinc.

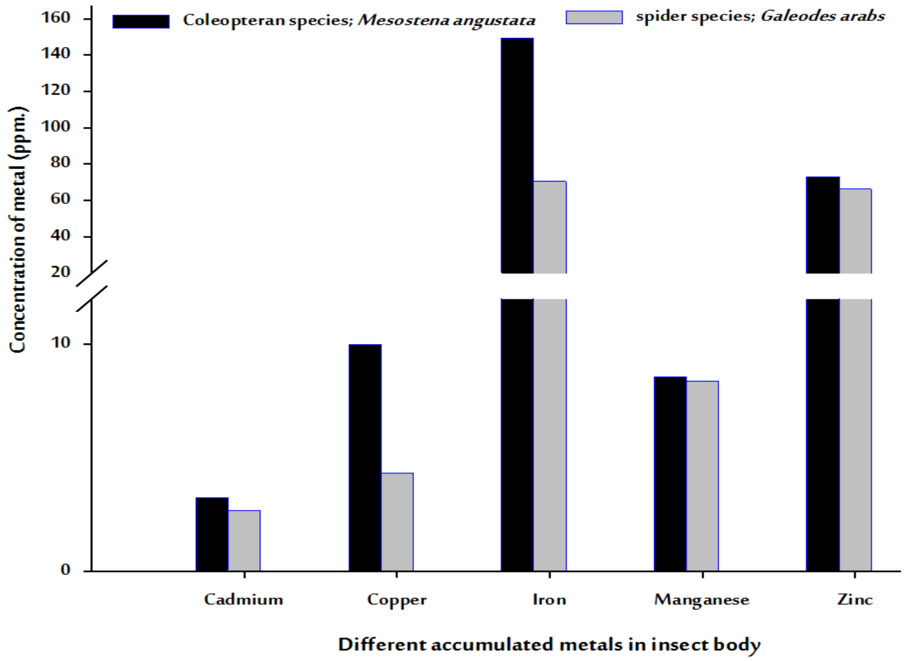

Fig. 3: Variation in the bioaccumulation of different heavy metals between coleopteran species, *Mesostena angustata* and spider species, *Galeodes arabs* in El Sadat industrial region, El Sadat city, Menofia Governorate, Egypt.

There were highly significant differences between different industrial zones investigated, where the lowest level of heavy metal accumulation was detected in the natural environment zone, and the highest levels were found in heavy industries followed by the medium industries zone. However, *M. angustata* revealed more capacity for heavy metal accumulation than the spider species (Fig. 4).

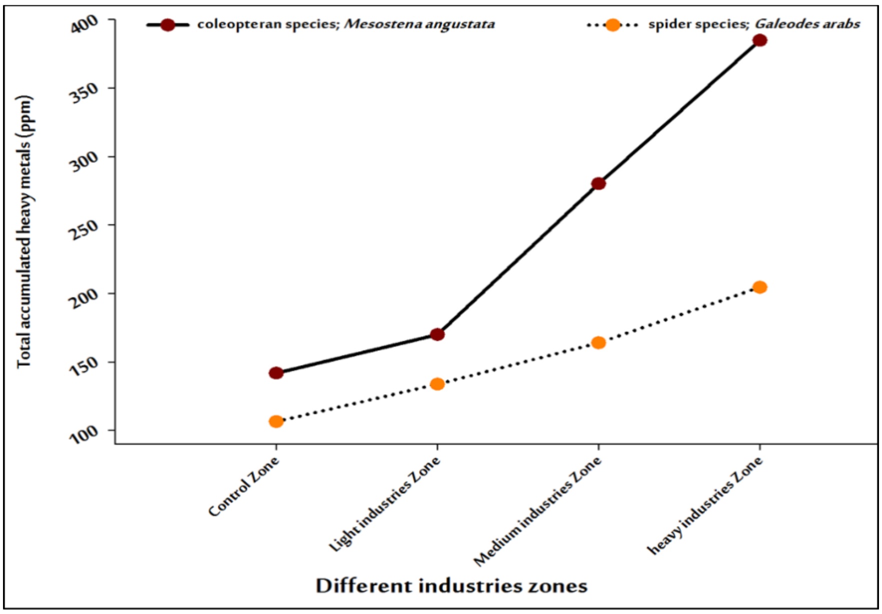

Fig. 4: Spatial variation in the concentration of total accumulated metals for both species as bioindication index between different levels of industries, in El Sadat industrial region, El Sadat city, Menofia Governorate, Egypt.

Spatial variation in the concentrations of the tested heavy metals at the different industries zones as investigated in this study for *M. angustata* revealed that major accumulated elements were; iron, zinc, copper and manganese (Fig. 5A). Iron and zinc were the most accumulated metals in the medium at the heavy industries zones. The heavy industries zone particularly showed higher accumulation levels of heavy metals, it recorded (251.71 ± 1.056 ppm) for iron; (102.0 ± 1.75 ppm) for zinc; and (14.46 ± 0.32 ppm) for copper. The spatial variation of these metals in *G. arabs* (Fig. 5B), showed that this species accumulated a higher average of accumulated metals as given below: iron (92.63 ± 0.45ppm) > zinc (88.77 ± 0.45 ppm) > manganese (12.32 ± 0.133 ppm) > copper (7.5 ± 0.28 ppm) at heavy industries zone, followed by a zone of medium industries. Cadmium was the lowest accumulated element in both tested species.

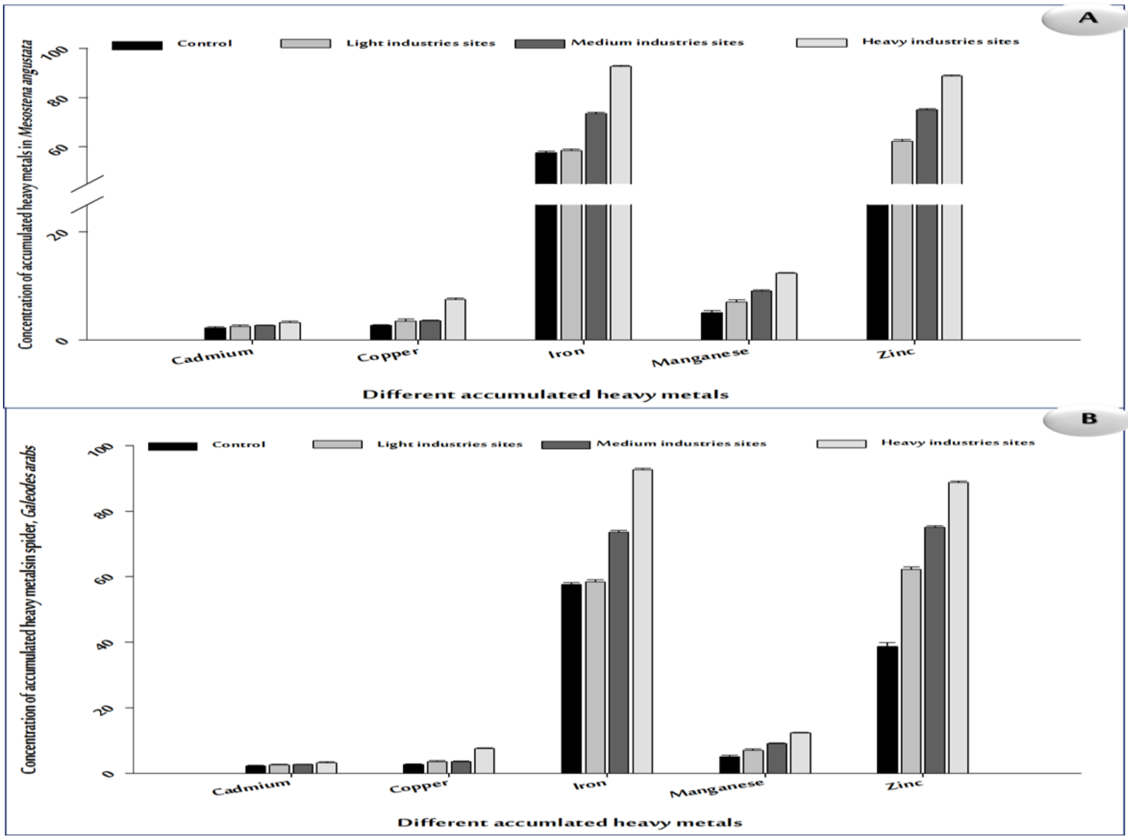

Fig. 5: Spatial variation of the concentration of different heavy metals (ppm) for (A), *Mesostena angustata and* (B), *Galeodes arabs* at different levels of industries, El Sadat industrial region, El Sadat city, Menofia Governorate, Egypt.

Pollution Index:

The calculated metal pollution index (MPI) clarified the seasonal and spatial variations in the average metal pollution degree (Table **2**). Generally, the average value of MPI in *M. angustata* was high during summer (32.65) and winter (15.73) than those during the spring and autumn seasons. The highest value of the MPI index was recorded in the zone of heavy industries (25.73) followed by the zone of medium industries (15.73) and light industries (10.93) as compared with the minimum value recorded in the natural environment zone. Similarly, the MPI index showed the same pattern for *G. arabs.* It recorded high average value during summer (18.425) and winter (12.83) than those of the spring and autumn seasons. The highest value of the MPI index (Table 2) was recorded in the heavy industries zone (19.73) followed by the medium industries zone (13.95) and light industries zone (10.35) as compared with the minimum value recorded in the natural zone.

Table 2: Spatial and seasonal variation in the metal pollution index (MPI) by beetle species, *Mesostena angustata* and spider species, *Galeodes arabs* at different levels of industries, El Sadat city, Menofia Governorate, Egypt.

Zone	Metal pollution index (MPI) values in *Mesostena angustata*					Metal pollution index (MPI) values in *Galeodes arabs*				
	Spring	Summer	Autumn	Winter	Mean	Spring	Summer	Autumn	Winter	Mean
Cont.	1.8	21.7	6.4	6.4	9.075	1.8	10.6	4.9	3.4	5.175
Light indust.	5.3	23.6	4.2	10.6	10.925	5.3	14.9	10.7	10.5	10.35
Medium indust.	6.8	34.9	4.8	16.4	15.725	6.8	20.9	12.7	15.4	13.95
Heavy indust.	14.1	50.4	8.9	29.5	25.725	14.1	27.3	15.5	22.02	19.73

See footnote table 1.

Enzymatic Biomarkers:

Spatial and temporal variation of enzymatic activity revealed an extremely significant variation either in the coleopteran species, *M. angustata*, or spider species, *G. arabs*, throughout the study period.

As shown in (Table 3 and Figure 6), the enzymatic activities of both tested species were significantly affected in all the investigated seasons at different levels of industrial impacts. The seasonal variation of LDH in *M. angustata* (Table 3) ranged from (15.0 ± 2.9 µg/min/mg protein) during spring in the natural environment to reach about (107.0 ± 1.2 µg/min/mg protein) during summer in the zone of heavy industries (Fig. 1a, 6), while in *G. arabs* it ranged between 12.0 ± 0.5 µg/min/mg protein during autumn for the natural environment to a high of (468.0 ± 1.2 µg/min/mg protein during summer in the zone of heavy industries (Fig. 2a, 6). The lowest activity of AChE in both tested species was recorded during autumn in the natural environment (18.96 ± 0.033 µg/min/mg protein for *M. angustata* and 11.0 ± 0.58 µg/min/mg protein for *G. arabs*), while the highest recorded AChE activity was during summer (46.7 ± 2.02 µg/min/mg protein for *M. angustata*) and autumn (47.0 ± 0.57 µg/min/mg protein for *G. arabs*) in high industries zone (Fig. 1b and 2b, 6).Seasonally, the activity of LDH and AChE in both tested species was shown in graph (Fig. 6). For *M. angustata*, the highest activity values were recorded during summer (LDH, 77.0 ± 1.44 and AChE, 41.67 ± 1.66 µg/min/mg protein) and winter (59.0 ± 0.62 and 33.67 ± 0.66 µg/min/mg protein, respectively) mostly related to their activities at the zones of heavy industries and medium industries respectively (Fig. 1a & b, 6). While, *G. arabs* recorded the highest activity during summer (191.89 ± 1.2 µg/min/mg protein) for LDH (Fig. 2a & b, 6).

Table 3: Seasonal and spatial variation in enzymatic activities of lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) of both sampled species; *Mesostena angustata & Galeodes arabs* at different zones of industries throughout the study period in El Sadat industrial region, Menofia Governorate, Egypt.

Enzyme	Zone	Enzyme activity (µ/mg) in *Mesostena angustata*				Enzyme activity (µ/mg) in *Galeodes arabs*			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
lactate dehydrogenase (LDH)	Cont.	15.0 ± 2.9^{d}	41.0 ± 0.58^{d}	24.0 ± 0.58^{d}	30.3 ± 0.9^{c}	23.7 ± 1.8^{c}	95.5 ±1.2^{c}	12.0 ± 0.57^{c}	46.0 ± 0.57^{d}
	Light indust.	18.0 ± 0.58^{cd}	55.0 ± 0.58^{c}	28.0 ± 0.58^{c}	49.0 ± 0.58^{b}	30.0 ± 2.9^{b}	100.33 ± 0.9^{b}	18.0 ± 0.57^{b}	51.3 ± 0.9^{c}
	Medium indust.	22.0 ± 0.58^{b}	105.83 ± 0.2^{b}	55.0 ± 0.58^{b}	78.0 ± 1.5^{a}	38.0 ± 0.001^{a}	104.0 ± 0.57^{ab}	20.0 ± 0.57^{b}	129.0 ± 0.6^{b}
	Heavy indust.	25.0 ± 1.2^{a}	107.0 ± 1.2^{a}	70.0 ± 0.58^{a}	78.7 ± 0.67^{a}	44.0 ± 0.57^{a}	468.0 ± 1.2^{a}	21.3 ± 0.8^{a}	107.0 ± 1.2^{a}
Acetylcholinesterase (AChE)	Cont.	21.0 ± 0.58^{c}	31.0 ± 0.577^{c}	18.96 ± 0.033^{c}	25.7 ± 0.89^{d}	14.0 ± 0.57^{c}	21.67 ± 0.88^{b}	11.0 ± 0.58^{c}	19.0 ± 2.3^{c}
	Light indust.	22.0 ± 1.15^{b}	44.0 ± 1.15^{b}	28.0 ± 0.58^{b}	29.0 ± 0.57^{cd}	18.0 ± 0.57^{bc}	31.0 ± 0.57^{a}	12.3 ± 0.33^{c}	29.0 ± 0.58^{b}
	Medium indust.	32.0 ± 1.15^{a}	45 ± 2.88^{ab}	29.0 ± 0.577^{b}	34.0 ± 0.58^{b}	23.0 ± 0.57^{b}	31.0 ± 1.2^{a}	22.0 ± 1.2^{b}	31.3 ± 0.3^{b}
	Heavy indust.	33.0 ± 1.73^{a}	46.7 ± 2.02^{a}	34.0 ± 0.58^{a}	46.0 ± 0.58^{a}	40.0 ± 2.88^{a}	38.0 ± 0.57^{a}	47.0 ± 0.58^{a}	37.0 ±1.2^{a}

See footnote table 1.

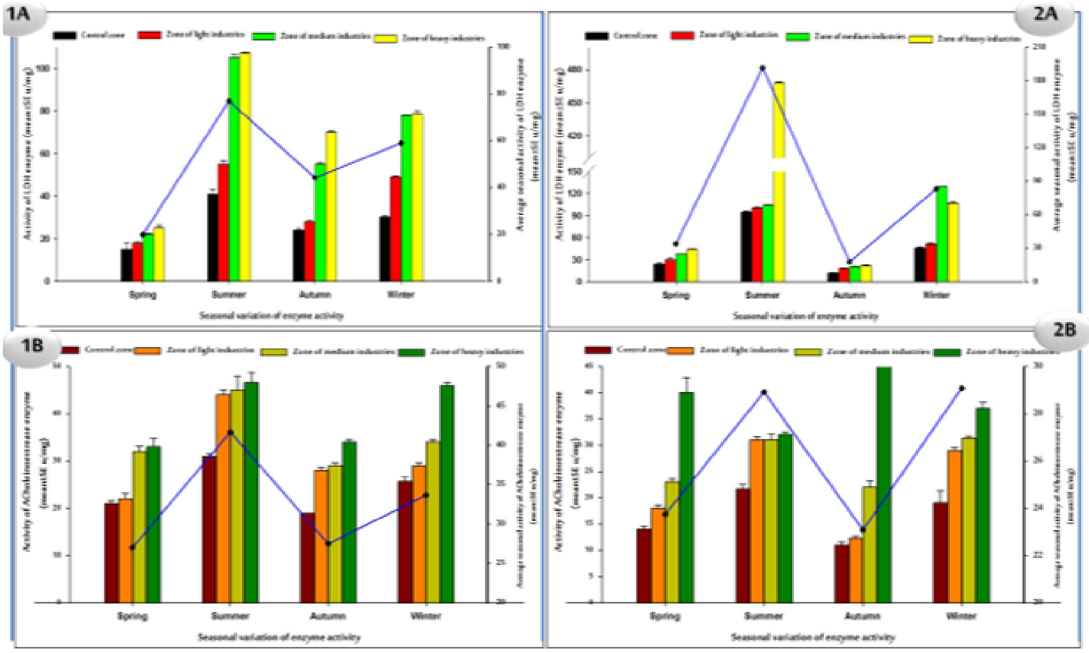

Fig. 6: Seasonal and spatial variation in enzymatic activities of lactate dehydrogenase (LDH) and Acetylcholinesterase (AChE) in both tested species; *Mesostena angustata* (1a, b) and *Galeodes arabs* (2a, b) at different levels of industries zones throughout the study period in El Sadat industrial region, Menofia Governorate, Egypt.

Interaction

Pearson correlation data (Table 4) exhibited a significant positive correlation between increased industrial impact and the accumulated mass of heavy metals (THMs) at r= 0.756, $P< 0.01$. Besides, the activity of both investigated detoxification enzymes was increased with the totally accumulated heavy metals with a positive correlation between both enzymes. Moreover, AChE activity was positively correlated with the accumulated metals which were; iron, zinc, manganese, copper and cadmium. Meanwhile, the activity of LDH was positively correlated with that of manganese and zinc accumulated metals. However, the activity of both tested enzymes was highly significantly correlated ($P< 0.001$).

Table (4). Pearson correlation coefficient (r) between different variables clarifies the relationship between different levels of industries, total accumulated metals (THMs), different metals and enzymatic biomarkers (lactate dehydrogenase (LDH) and acetylcholinesterase (AChE).

	Industries levels	THMs	Cadmium	Iron	Manganese	Copper	Zinc	LDH	AChE
Industries levels	1								
THMs	0.756**	1							
Cadmium	0.803**	0.954***	1						
Iron	0.634*	0.982***	0.894***	1					
Manganese	0.951***	0.734**	0.830**	0.598*	1				
Copper	0.599*	0.936***	0.949**	0.930**	0.624*	1			
Zinc	0.953**	0.810**	0.889	0.685	0.968***	0.704**	1		
LDH	0.761**	0.266	0.377	0.131	0.746*	0.158	0.608*	1	
AChE	0.887***	0.831	0.936***	0.733**	0.884**	0.833**	0.912***	0.615*	1

*** Correlation is significant at 0.001 level (2-tailed); ** Correlation is significant at 0.01 level (2-tailed); and *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Ecotoxicological studies have attracted great interest worldwide in the last few decades. Seeking a successful trend in the assessment of anthropogenic environmental pollutants pointed to the detection of accumulated heavy metals in surrounding organisms. Invertebrates are considered useful bioindicators to estimate the impact of heavy metals accumulation particularly if there was direct contact with the metalliferous soil or metal-rich food in their surrounding environment (Gall *et al.*, 2015).

In this study, two species were selected as bioindicators for heavy metals accumulation; the first one resembles the coleopteran family (Tenebrionidae), which is a good bioindicator for soil pollution due to its wide variety of habitats. We can find it almost in all the biogeographical regions- in addition to its anthropogenically-induced landscape modification (Osman *et al.*, 2015). To give a comprehensive picture of the different metal-contaminated zones and their surrounding environments, we tried to study the route of heavy metals throughout the food chain. To do so, the second species was the spider *G. arabs* which was selected as a bioindicator of heavy metals accumulation in food. *G. arabs* is a carnivorous species that possess specific feeding characteristics that allow high concentrations of biogenic metals tolerance in their bodies due to metal excretion and storage than those of their prey (Wilczek and Babczyn´ska 2000). Therefore, spiders are known as potent biological indicators of heavy metal contamination (Maelfait, 1996).

Herein, the spatial and seasonal variation in the accumulated heavy metals in the whole-body tissue homogenate was investigated. Detection of heavy metals in the whole-body tissue homogenate is preferred to detection in a certain organ due to more availability of heavy metals and accuracy (Cain *et al.* 1995). Iron and zinc recorded higher levels of heavy metal accumulation than the other tested metals such as cadmium, which may be attributed to their abundance in the surrounding environment in soil and plants. These results were in agreement with Jelaska *et al.* (2007) who attributed the detection of heavy metal concentrations in ground beetles to their abundance in their inhabited soil. Similarly, Azam *et al*. (2015) attributed the increased heavy metals accumulation level in the terrestrial insects which inhabited the industrial zones to the elevated levels of accumulated heavy metals in the surrounding soil.

Copper and manganese were accumulated in low concentrations than iron and zinc for both *M. angustata* and *G. arabs*. For *T. duplicate,* Gambrell *et al*. (1991) reported increased soluble concentrations of cadmium and copper in insects that inhabited brackish or marshy soil with increased salinity. Elimination of heavy metals such as cadmium, zinc and copper was generally low in spiders compared to other invertebrates. In the present study, higher accumulation rates of heavy metals in *G. arabs* were attributed to the high predation rate of preys with elevated metal contents in their bodies such as collembola, dipteran larvae and other spiders (Wilczek and Babczyńska, 2000; Jung and Lee 2012). However, the metal body burden of spiders may be related to the available metal contents in the soil.

By comparing the seasonal and spatial accumulation of heavy metals in both species, *M. angustata* was more susceptible to the accumulation of metals than *G. arabs*. This may be attributed to the direct contact of tenebrionid species with the surrounding environment when compared with the carnivorous spiders that accumulate heavy metals throughout their prey only. In the same context, Zhou *et al.* (2019) confirmed that insects that inhabit soil contain much more heavy metal concentrations than longicorn and cicada insects which could potentially pose a negative impact on the predators along food chains.

The natural environment showed the lowest concentrations of bioaccumulated heavy metals during the study period. In contrast, the heavy industries zone showed a significant increase in the concentrations of iron, zinc, copper, and manganese across all seasons when compared with other zones with less industrial loads. It could be due to the nature of the various companies in the industrial zones es and they include; metallurgical, textile, petrochemicals, electrical, marble and plastics companies. Bioindication of spatial variation of heavy metals accumulation by both tested species clarified that there was a significant relationship between the zones with different industrial loads and the concentrations of accumulated heavy metals., This may be attributed to the variability in the levels of heavy metals accumulation, environmental degradation, contamination, and the distance between pollution sources and sampling sites (Rabitsch, 1997). Similar results were reported by Heliövaara and Väisänen (1990) on adults and immature stages of the European pine sawfly; and Soliman and El-Shazly (2017) on the grasshoppers which inhabited soils and plants in the industrial zones.

Metal pollution index (MPI) is one of the important pollution indices, which provides a status of the environment towards any environmental impact and its effect on the ecosystem Mokhtar, Aris, *et al.* (2009). In the current study, spatial variation of MPI revealed its increment upon the industry levels. It may be arranged as follows: heavy industries >medium industries >light industries. This arrangement may be due to the mass accumulation of heavy metals and nearness to effluents as explained by some earlier workers (Rodríguez-Barroso, Benhamou, et al. 2010).

The important detoxification role of AChE and LDH enzymes towards heavy metals accumulation or pollutant degradation is well-known (Siscar *et al.*, 2014). Detoxification enzyme measurement is an important biomarker method used in ecotoxicological studies as a bioindicator to determine the stress level due to exposure to heavy metal pollution, insecticide usage, or any other types of stressors. Detoxification enzymes play an important role to protect cells from oxidative damage (Wilczek *et al*., 2008). In this study, there was a positive correlation between pollutants (industry levels) and the investigated enzymes in both tested species. This relationship depends upon the mechanism that deals with additional stressors throughout heavy metals accumulation.

Acetylcholinesterase a neurotransmission enzyme that increases additional stress - by hydrolysis of acetylcholine into choline and acetic acid at the cholinergic synapses and neuromuscular junctions- makes the postsynaptic membrane in a state of permanent stimulation (Peña-Llopis *et al.*, 2003). Herein, the spatial and seasonal variations in the levels of AChE were significantly different throughout the study period. The highest level of this enzyme was reached during summer in the zone of heavy industries. These results are in agreement with Hendrickx *et al*. (2003) who considered the elevated levels of detoxification enzymes in spiders toward heavy metals stress as strong physiological reactions; Sun *et al.* (2008) who recorded the increased activity of detoxification enzymes in *Spodoptera litura* toward the accumulation of xenobiotics; and Qin *et al.* (2012) who found that spiders in highly polluted areas developed some compensatory mechanisms which caused increase synthesis of enzyme molecule and high AChE activity. Furthermore, Butt and Aziz (2016) stated that bioaccumulation of high levels of copper remarkably impacts the activity of detoxification enzymes in the wolf spider, *Pardosa oakleyi*.

Lactate dehydrogenase -a glycolytic enzyme involved in carbohydrate metabolism- has been used as an indicator of chemical stress (Diamantino *et al.*, 2001). Spatial and seasonal variations in LDH levels in both tested species were investigated. Seasonally, the highest activity of LDH was recorded in the summer season. There was a gradual increase in LDH activity with industry levels, the highest recorded activity was in the zone of heavy industries. The obtained results revealed high levels of LDH activity in spiders than those of tenebrionid beetles. The survival of organisms in polluted environments is mainly due to metal detoxification. Spiders exhibited much more resistance to the bioavailable metals by increasing levels of detoxification enzymes above the normal range, (Zvereva *et al*. 2003). Contrary, Arshad *et al.*, (2002) found that LDH activity was decreased in *Culex* sp. with highly dramatic ratios as a response to insecticides stress after treatment with DDT, Malathion and Cyfluthrin. Nathan *et al.* (2005) showed that treatment with azadirachtin decreased the LDH activity in the midguts of *S. litura*; and Zibaee *et al.*, (2008) found similar results for the rice striped stem borer treated with diazinon.

Pearson correlation showed the positive relationship between increased industries level and the average of total accumulated heavy metals, in particular, iron, zinc, copper and manganese. Additionally, the bioaccumulation of heavy metals in tested species led to the significant activity of detoxification enzymes to protect their body constituents.

CONCLUSION

In conclusion, tenebrionid beetle (*Mesostena angustata)* and carnivorous spider (*Galeodes arabs*) were selected to be investigated as bioindicators of heavy metals pollution and to evaluate the industrial impact in three main zones representing three levels of industries which were; the light, medium and heavy industries. The three main classes of industries were selected as the study sites in the El Sadat industrial region, Menofia, Egypt. Heavy metals bioaccumulation, AChE and LDH activities were assessed in both tested species across four successive seasons (Winter, Summer, Autumn and Spring). Significant variations in seasonal and spatial bioaccumulation of heavy metals by both tested species particularly in the summer and winter seasons among the heavy industries zone and medium industries zone were observed. The major accumulated elements were; iron and zinc in both tested species. Increased activity of AChE and LDH was related to the bioaccumulation of heavy metals, particularly in the heavy and medium industries zones. In inference, the tested species may be considered promising bioindicators of heavy metals pollution which could help in monitoring industrial heavy metals accumulation.

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ARABIC SUMMARY

الاستدلال الحيوي للخنافس والعناكب في دراسة السمية البيئية لتأثير المعادن الثقيلة وتفاعلها مع إنزيمات إزالة السموم.

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حديثا، أصبح الاستدلال بالكائنات الحية وخصوصا الخنافس والعناكب اتجاه دقيق وسريع في تقييم السمية البيئية. حيث تم اختيار وتجميع حشرة خنفساء (*Mesostena angustata*) وعنكبوت (*Galeodes arabs*) كنوعين مختلفين في الطبيعة الغذائية كمؤشرات بيولوجية بعد دراسة مسبقة لتقييم الأثر الصناعي في منطقة السادات الصناعية، المنوفية، مصر. ونظرا لتنوع الصناعات فى تلك المنطقة فقد تم اختيار ثلاث مناطق متنوعة طبقا لفئة الصناعات المسجلة هناك فمنها الصناعات الخفيفة والمتوسطة والثقيلة مقارنة بالبيئة الطبيعية. حيث تم تقييم التراكم الحيوي للمعادن الثقيلة والنشاط المتفاعل لأنزيمات إزالة السموم من خلال انزيمى أسيتيل كولينستريز واللاكتات ديهيدروجينيز في كلا النوعين خلال أربعة مواسم متتالية. كما أبرزت النتائج اختلافات معنوية فى تقييم التراكم البيولوجي الموسمي والمكاني للمعادن الثقيلة فى كلا النوعين، فكان فصل الصيف هو أعلى المواسم تراكما في المعادن الثقيلة في منطقة الصناعات الثقيلة، تليها الصناعات المتوسطة وهذا ما أكد من خلال مؤشر التلوث المعدني. كما أوضحت النتائج تواجد مكتسح لعنصري الحديد والزنك كعناصر متراكمة على عكس تواجد الكادميوم في كلا النوعين. كما أظهرت النتائج أن نشاط انزيمى أسيتيل كولينستريز واللاكتات ديهيدروجينيز المتزايد مرتبط ارتباطا ايجابية مع تراكم المعادن الثقيلة داخل الجسم، لا سيما في منطقة الصناعات الثقيلة. في الاستدلال، يمكن اعتبار الأنواع التي تم تقييمهما مؤشرات حيوية واعدة لتلوث المعادن الثقيلة والتي يمكن أن تساعد في مراقبة تراكم المعادن الثقيلة الصناعية.