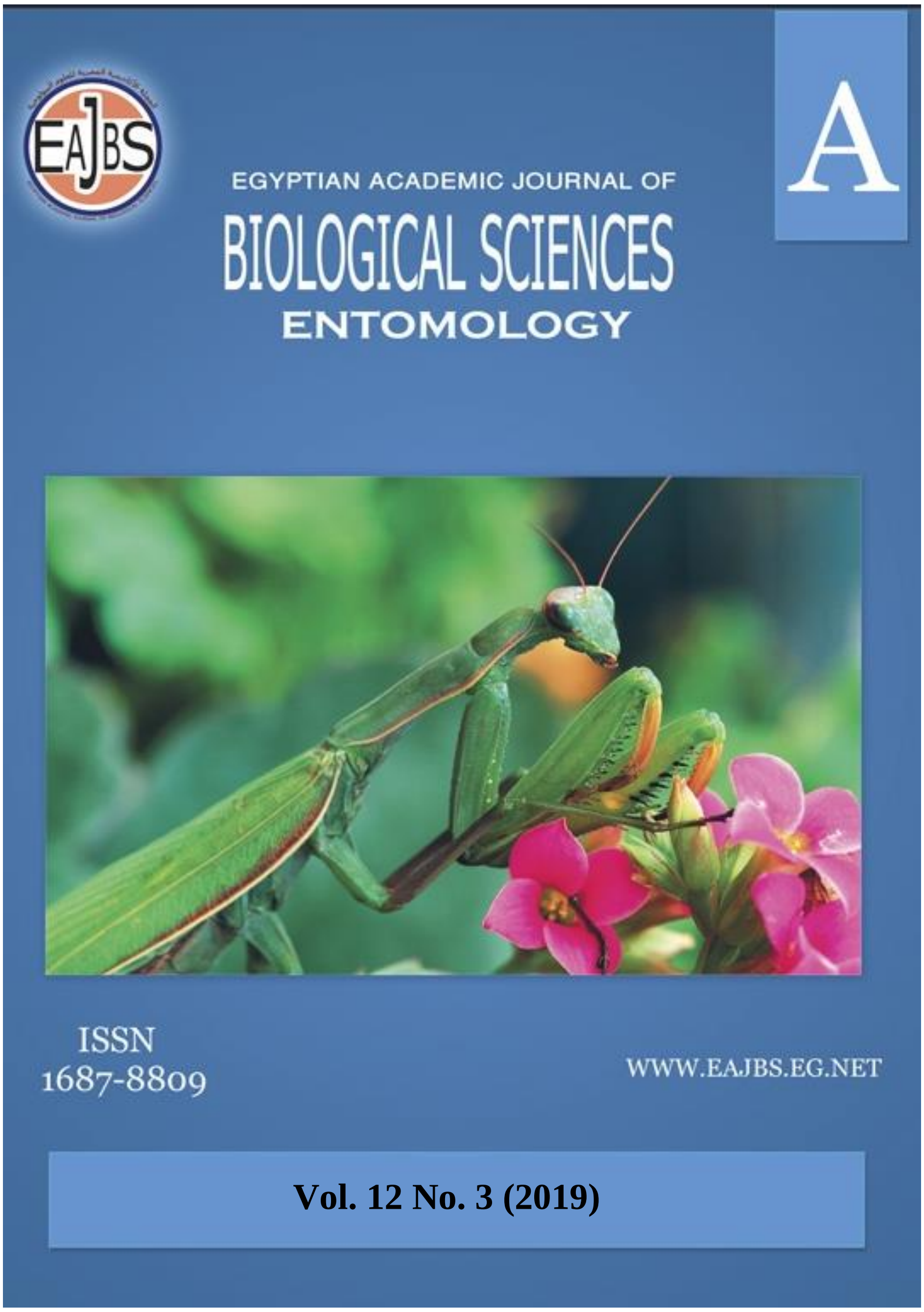

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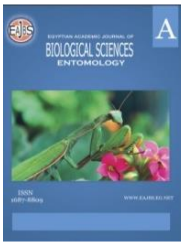

Industrial Pollution Evaluation through Enzymatic Biomarkers at Different Localities of El-Sadat Industrial City, Menofia, Egypt.

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ABSTRACT

Industrial pollution is one of the most dangerous that is a continuous threat for humanity. Evaluation of that pollution is the main aim of the current study through the detection of the accumulated heavy metals in its surrounding habitats mainly soil and flora as well as the response of insects towards this pollution. Therefore, seasonal collection of three different insect species were performed in addition to soil and the common plant; *Zilla spina*, at different sites of El-Sadat industrial city, Menofia, Egypt for four successive seasons during 2016 to 2017. The Acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) extracted from the homogenate of the chosen insects were used as biomarkers for heavy metals pollution. Also, heavy metal contents in plant and soil samples were analyzed. Results showed varied activities for the investigated biomarkers depending on the study sites, providing a representative picture to the environmental state of that areas.

INTRODUCTION

Since the eighteenth century, industerial revolution and technological advancement were the major human impacts on natural ecosystems (Ives and Cardinale, 2004). The main dangers of this intensification have arisen from the emission of heavy metals and other toxic elements, which cause some irreversible and uncontrollable consequences of global serious environmental pollution. Therefore, negatively impact on wildlife populations and causes the appearance of destabilizing impacts on ecosystems (Kappeler, 1979; Sericano *et al.*, 1995; Twardowska, 2004; Echols *et al.*, 2009). Heavy metals are classified as the most dangerous pollutant of anthropogenic environmental pollutants not only for their toxicity to the biota but also for not chemically or biologically degradable, persistence in the environment and accumulative element in any ecosystem even in human body (Lee *et al.*, 2006; European Environment Agency, 2011; AESAN, 2012; Guo *et al.*, 2012; Koz *et al.*, 2012). Not all heavy metals are dangerous elements but there are biologically essential metals which play important role in a biological system in human being on opposite to non-essential elements which are toxic to living organisms even at quite low concentrations (Alkan *et al.*, 2015; Ndimele *et al.*, 2017).

Soil consider a bed reservoir to pollutants, an accumulator of heavy metals, and a good indicator of environmental quality (Danesino, 2009). A large part of the research on heavy metals has targeted on their accumulation and persistence in the soil, their translocation via the food chain and directly related with the sustainability of ecological systems (Wuana and Okieimen, 2011; Opaluwa *et al.*, 2012). Many authors evaluated the industrial pollution throughout the accumulation of heavy metals in soil at different industrial areas (An, 2004; Cui *et al.*, 2004; Wuana and Okieimen, 2011; Opaluwa *et al.*, 2012; Gbarato Oliver *et al.*, 2015) and concluded the efficiency of soil as powerful tracers for monitoring industrial emission.

Plant in another facet the potential to excessively be absorbed and taken heavy metals from soil later become toxic (Monni *et al.*, 2001) and transported via food chain (Wuana and Okieimen, 2011; Opaluwa *et al.*, 2012). Plant consider an "early- warning" signal of pollution-induced stress symptoms due to the accumulation of heavy metals especially in the tissues and fluids of plants (Białońska and Dayan, 2005). Therefore, it appears very promising for the removal of pollutants from the environment and may be used as bioindicators to determine the level of pollution (Garbisu and Alkorta, 2003; United Nations Environment Programme (UNEP), 2010).

Among the consumer trophic level in food chain, insects which accumulate toxic environmental compounds in their tissues and organs (Introna *et al.*, 2001). Meanwhile, finding of the accumulation of metals concentration is more accurate and higher in the whole body than a single organ in insects (Cain *et al.*, 1995). Among the famous and distributed insects in any habitat in addition to widely monitoring the accumulation of heavy metals were ants (Belskii and Belskaya, 2013; Skaldina *et al.*, 2018), beetles (Bednarska *et al.*, 2013; Osman *et al.*, 2015; Zhou *et al.*, 2019), and grasshopper (Del Toro *et al.*, 2010; Mustafa and El-Shazly, 2017) they consider as good bioindicator for metal pollution especially in industrial areas.

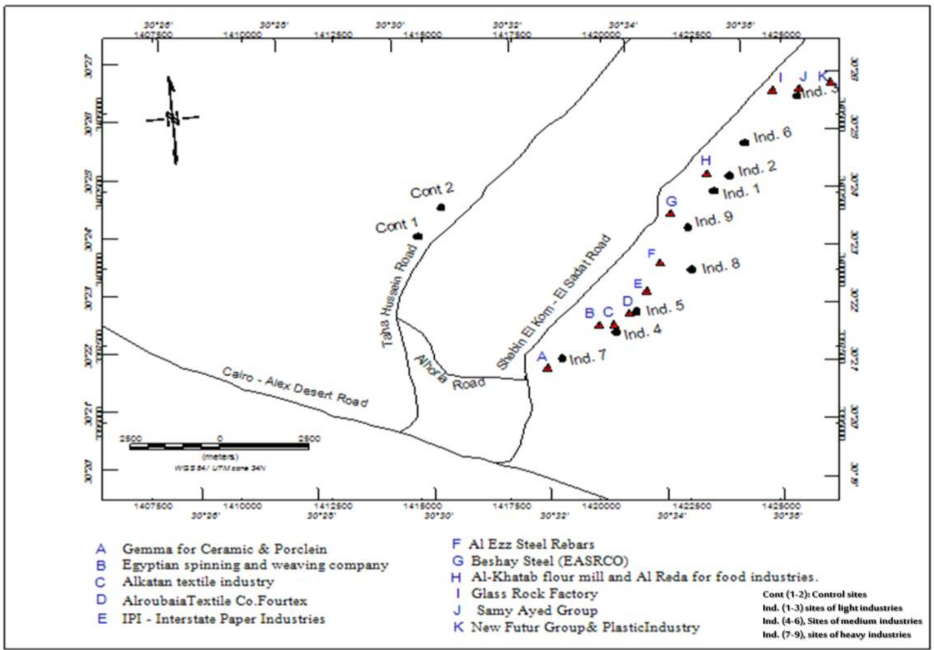
An early sensitive and responsive tool and widely used in the assessment of environmental contamination are enzymes because of their efficiency in degradation of different xenobiotics and its antioxidant activity in the protection of important molecules like DNA, RNA and proteins Augustyniak *et al.*, 2005; Wilczek *et al.*, 2008; Domingues *et al.*, 2010; Praet *et al.*, 2014)**.** (AChE) has been widely studied and employed as a biomarker in invertebrate and vertebrate species to detect exposure to chemicals in natural ecosystem (Lavado *et al.*, 2006)**.** In addition, Lactate dehydrogenase (LDH) is an important glycolytic enzyme, which can be used to indicate exposure to chemical stress present in virtually all animal tissues (Kaplan and Pesce, 1996).

The current study was conducted for investigation the ecotoxicological impact of industrial pollution on the surrounding ecosystem throughout exploration of heavy metals in soil and flora as the bed reservoir in habitat, as well as different insect species during detection of the activity of the detoxification enzymes in selected insect species as biochemical biomarkers and responsive tool of species towards pollution.

MATERIALS AND METHODS

Study Area:

Menofia Governorate is one of the biggest Governorates in Egypt. It is situated at the central Delta region and extends from the middle to south of Nile Delta; between Damietta (Dumyat) and Rosetta (Rashid) branches of Nile River. The largest industrial city in Menofia Governorate, El Sadat city which located 93 km to the North-West (30.6°N, 30.22°E) from Cairo (El-Tahawy *et al.*, 2006). This city is surrounded by a 350 km^2 green belt, which has earned it a place in the top ten lists of environmentally friendly industrial cities in the Middle East (Ayyad, 2016). The main industries varies between light industries such as food and drinking industry and different companies for export and import products; medium industries as weaving, spinning & dyeing, building articles, food & beverage products ready-made clothes; and finally heavy industries as iron & steel, engineering & metal products, electronic & electric appliances, wood products & furniture, plastic products, chemicals, transport vehicles, paper products, poultry, industrial gases, textile, paper production and medical products (Sadat industrial zone web site, 2018). 11 localities were chosen to represent as much as possible the status of heavy metals' bioaccumulation per time along Governorate. 3 sites were representing each of industry, as well as two control sites outlying away from industrial localities. The coordinates of each locality (Map 1) was recorded using a hand-held Global Positioning System (Garmin, GPS III plus).



Map (1). Description of the studied area

Sampling and Preparation of Soil and Plant:

Soil samples were collected at a depth of 0-50 cm from different study sites and divided according to the type of industries; light with (B), medium industries (C), and heavy ones (D) in comparing natural control sites (A). These samples were transported to the laboratory for well air-dried and clean bagged the portion finer than 2 mm for further analysis of heavy metals, as mentioned by Carter and Gregorich (2008).

Protocol of estimation the heavy metals in soil was summarized according to Quevauviller *et al.* (1993) and Vercoutere *et al.* (1995) as the following, 0.5 g of dried soil was initially digested by 37% hydrochloric acid HCl: 65% concentrated nitric acid (HNO_3) with ratio 3:1 mixture for 24 h at room temperature. Then, hooting the samples were conducted at 90 °C in a fume cupboard then raised for adding an identical quantity (2.5 mL) of HCl and 30% H_2O_2 added to complete the digestion. The resultant mixture was heated again and then cooled to ambient temperature. The flask walls were washed with 10 mL of deionized water and the suspension was then filtered through Whatman filter paper (No. 41) in a volumetric flask, diluted to 50 mL, and stored in polyethylene bottles at 4 °C for later analyses. Concentrations of heavy metals in the soil samples were determined by atomic absorption spectrophotometer and expressed as ppm dry weight.

The heavy metals in plants, *Zilla spina* (Family: Cruciferae (Brassicaceae)) is the common species at almost study sites. Therefore, it is the most suitable choice for detection heavy metals in this region. Plant samples were separately collected at different localities and divided as previous soil sampling for clean preserved and pressed to dryness. Plant sample was grounded and powered then stored separately until analysis.

According to Estefan *et al.* (2013), about 0.5-1.0 g of dry and ground plant material pre-digested by adding 5 mL concentrated nitric acid and swirled carefully then and leave for about 6-8 hours. After pre-digestion, then digested on hot sand bath using 10 mL from di-acid mixture, which concentrated nitric acid and concentrated perchloric (70%) with 9:4 ratios, swirled carefully. Digestion was continued until the dense white fumes evolve and transparent white contents are left, allowed to cool, filtered through a resistant filter paper and transferred to 50 ml volumetric flasks, diluted to the mark with distilled water and stored in clean sterile plastic bottles until determination of the selected heavy metals using an Atomic Absorption spectrophotometer.

Sampling and Preparation of Species:

Seasonal sampling of insects were carried out using pitfall trap which is commonly used and an effective sampling technique (Andersen, 1991; Hoffmann *et al.*, 2000; Lindsey and Skinner, 2001; King and Porter, 2005) over four successive seasons; autumn, 2016 to autumn, 2017 at different study sites in El-Sadat industrial city. The collected specimens were identified to the species level whenever possible.

After that, *Cataglyphis savignyi* (Hymenoptera: Formicidae) and *Adesmia* sp. (Coleoptera: Tenebrionidae) in addition to detection of metal accumulation in herbivorous insects–*Chrotogonus homalodemus*, (Orthoptera, Acrididae) were chosen for detection the antioxidant enzymes (AchE& LDH) towards the accumulation of heavy metals as the following. Whole body insects were weighed and then homogenized in a saline solution (1 gm. of tissue insect/1 ml saline solution 0.7 %) using homogenizer for 2 min. The homogenates were then ice-centrifuged at 4000 r.p.m for 15 min. The supernatant was used directly or frozen until the use for the measurement of each enzyme activity. Three replicates were used for these measurements at each one.

According to the technique adopted by Ellman *et al.* (1961), samples were assayed using kit for determining the activity of AchE concentrations by company of Quimica Clinica Aplicada S.A., Egypt. Meanwhile, the activity of Lactate Dehydrogenase Activity (LDH) was determined using a kit purchased by Biosystem reagents. The enzyme was then measured at 340 nm by spectrophotometer according to the method of Tietz (1999).

Data Analysis:

Seasonal variation of heavy metals and the activity of enzymes were represented in mean± standard error (Se). One-way ANOVA was used for detection the significant difference between different testified groups. Different letters were used for expressing the significant groups, dependent Post-hoc analysis by Least Significant Difference (LSD) test and Tuckey's test. According to Snedecor and Cochran (1982), a statistically significant between the testified groups was clarified at $P<0.05$. Meanwhile, Pearson correlation was used for detection the relationship between each of the following: different elements of heavy metals, different types of industries, and the activity of enzymes. The strength of relationship is clarified throughout correlation coefficient (r^2). Its value is closer to 1 or -1 are the strongest positive or negative correlations, respectively. Values above about ± 0.4 are probably significant and 0 is no linear correlation. Unfortunately, the significance of the correlations is not given in the output (Galton, 1886; Pearson, 1895; Stigler, 1989). SPSS (Statistical Package for Social Sciences, Inc.) version 20.0 for Windows 7 was used for detection the data analysis and Sigma plot version 11 for plotting data.

RESULTS

Heavy Metals in Soil and Flora:

Accumulation of heavy metals in soil and flora was measured at different industrial sites in Menofia Governorate (Table 4). The results clarified that the highest concentration level of heavy metals in soil were detected at the sites of heavy industries (D) following with medium industries one (C) mainly, the concentration of iron (1238.1± 61.3& 1160.4 ±5.8), copper (26.2 ± 0.64& 25.5 ± 0.001) and manganese (289.8 ± 0.01& 261.7 ± 0.01, respectively). Meanwhile, the highest concentration of zinc was attained at the sites of medium industries (C) following with heavy industries sites (D) (341.3 ± 75.4& 235.4 ± 4.4, respectively). The statistical analysis proved that the relationships between the before mentioned metals and study sites were statically which significant.

On the other hand, the accumulation of heavy metals in flora Table 4 revealed an obvious significant difference between this accumulation and the study sites. The highest heavy metals accumulation were detected at the sites of medium industries (C) following with heavy industries sites (D) mainly, the concentration of iron (1146.6 ± 17.8& 1099.2 ± 60.7) and zinc (309.7 ± 3.3& 306.7 ± 4.3). Finally, the highest manganese concentration level was (225.2 ±30.0 & 200.1 ± 57.7, respectively).

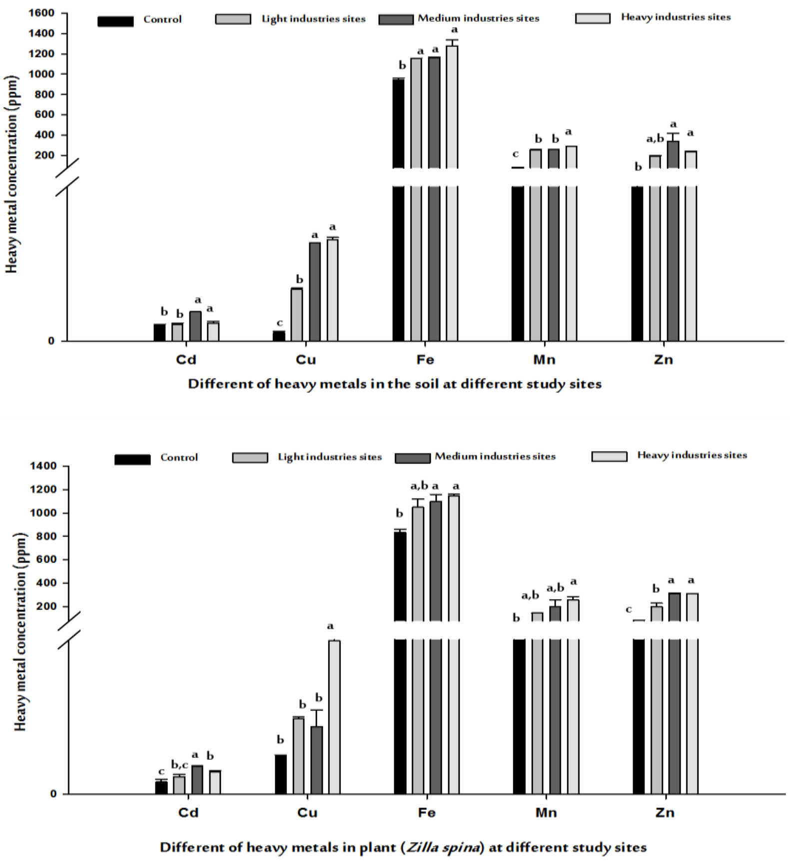


Fig. (1). Spatial variations in the values of heavy metals (ppm) of the soil and plant samples collected from different industrial sites throughout the current study in Menofia Governorate, Egypt. The given values are the means ± standard error. Post hoc test indicates that the different superscript letters are significantly different with Tuckey's-b test (Tukey's tested post hoc tests based on one-way ANOVA mean values of different diversity matrics at , $P < 0.05$). Note that, (a): not significantly different (P>0.05), (b): Significantly different (P<0.05), (c): Highly significantly different (P<0.01) respectively.

Seasonal Variation of the Activity of Enzymes:

Seasonal variation in the activities of (LDH) and (AchE) was detected in the cited species in Table (1). Summer recorded the highest activity of lactate dehydrogenase (LDH) at three different species (*Cataglyphis savignyi*, 118.58± 1.7u/mg; *Adesmia* sp., 142.0 ± 1.82u/mg; and *Chrotogonus homalodemus*, 119.0 ±1.3 u/mg). Meanwhile, spring (*Cataglyphis savignyi*, 23.13± 2.2 u/mg& *Chrotogonus homalodemus*, 24.13 ± 0.6 u/mg) represented the lowest season of its activity only in two species and autumn in third species (*Adesmia* sp., 19.0 ± 1.8u/mg).

As the accordance with the activity of lactate dehydrogenase, the activity of (AchE) at the cited species (*Cataglyphis savignyi*, 49.4±0.6u/mg; *Adesmia* sp., 56.7 ± 1.4u/mg; and *Chrotogonus homalodemus*, 60.0 ± 0.8u/mg) during summer. Oppositely, the lowest season in the activity of this enzyme was differed at cited species as the following (during spring in *Cataglyphis savignyi*, 19.0± 1.4 u/mg; during autumn in *Adesmia* sp., 16.75 ± 1.4u/mg; and during winter in *Chrotogonus homalodemus,* 36.7 ± 0.8 u/mg).

Table (1). Seasonal variation in the activities of lactate dehydrogenase (LDH) and (AchE) at different study period, El-Sadat city, Menofia Governorate.

Enzymes	Species	Study seasons			
		Spring	Summer	Autumn	Winter
LDH	*Cataglyphis savignyi*	23.13± 2.2^{ns}	118.58± 1.7***	53.83± 1.1***	31.75± 1.3***
	Adesmia sp	29.3 ± 1.82*	142.0 ± 1.82***	19.0 ± 1.82***	131 ± 1.82**
	Chrotogonus homalodemus	24.13 ± 0.6*	119.0 ±1.3***	112.0 ± 14.5***	37.5 ± 0.57***
AchE	*Cataglyphis savignyi*	19.0± 1.4***	49.4± 0.7***	35.5± 2.33**	27.48±1.1 ^{1**}
	Adesmia sp	17.0 ± 1.4***	56.7 ± 1.4***	16.75 ± 1.4**	23.75 ± 1.4**
	Chrotogonus homalodemus	38 ± 1.3 **	60.0 ± 0.8 ***	46.0 ± 0.5^{ns}	36.7 ± 0.8 **

The given values are the means ± standard error. Post hoc test indicates that the different superscript letters are significantly different with Tuckey's-b test (Tukey's tested post hoc tests based on one-way ANOVA mean values of different diversity matrics at , P < 0.05).While post hoc test indicates that *, **, and *** above are significantly different at P< 0.01, P< 0.001, and P< 0.0001 respectively, meanwhile ^{ns} is non-significantly different.

Spatial and Temporal Variation of Enzymes

1. Enzymatic Variation in *Cataglyphis savignyi:*

Figure (2) clarified the seasonal activity of both enzymes; (LDH) and (AchE) in *Cataglyphis savignyi*, at different study sites with significantly between study sites at P< 0.01and 0.001except the activity of lactate dehydrogenase (LDH) during spring at P >0.05. Where, an increment of the activity of lactate dehydrogenase (LDH) was clearly at sites of heavy industries with (D) following with sites of medium industries (C) (158±3.5& 108±1.2 u/mg, respectively) during summer. Control sites recorded the lowest activity of this enzyme during winter (10±1.7 u/mg). On the other hand, the activity of (AchE) showed the maximum activity at sites of heavy industries during summer (67±0.6 u/mg) and also, the minimal activity during spring (11±1 u/mg) there.

An extremely significant difference in the activity of both enzymes of *Cataglyphis savignyi* between sites of different industries at P< 0.01and 0.001. Throughout the annual study, an increment of the activity of lactate dehydrogenase (LDH) was clearly at sites of heavy industries with (D) following with sites of medium industries (C) (78.33±2.6 & 60.68±1.35u/mg, respectively). Oppositely, control was the lowest site (43.3±2.02u/mg) between the study sites. On the other hand, the activity of (AchE) showed the maximum activity at sites of medium and heavy industries (34.92±1.8& 34.3±1.78 u/mg, respectively) and its reduction was at control sites (25.4±0.6 u/mg).

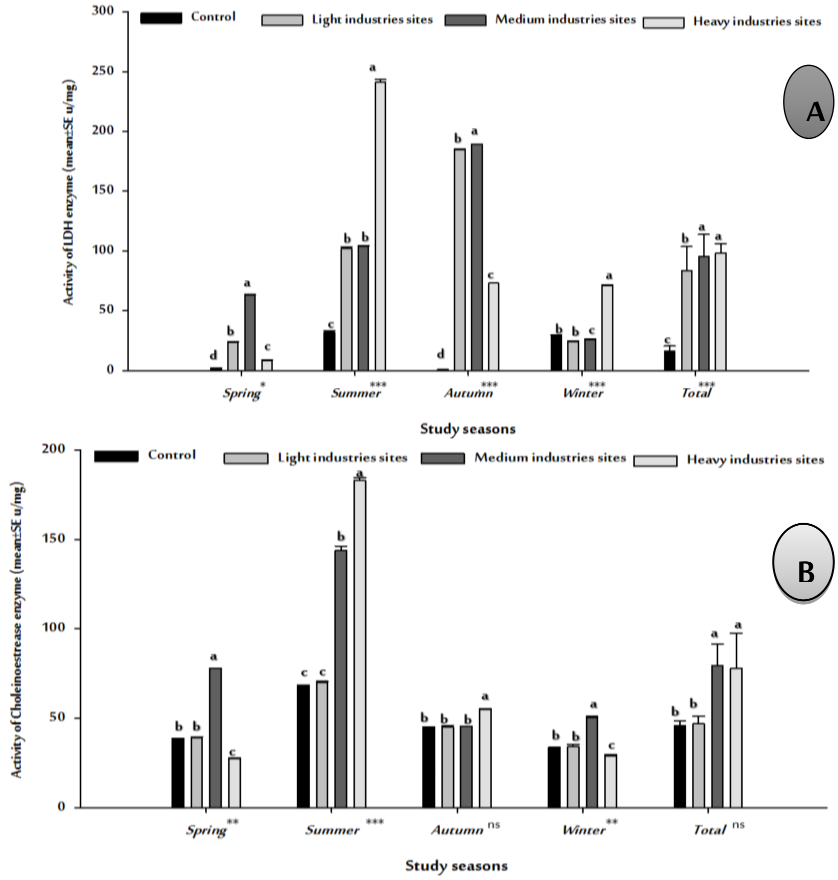


Fig. (2). Spatial and seasonal variation of the activity of LDH and AchE enzyme (mean±se u/mg) in *Cataglyphis savignyi* at different study sites throughout study period, El-Sadat city, Menofia Governorate. Post hoc test indicates that the different superscript letters are significantly different, while the symbols ^{ns}, *, **, and ***: see table (1); (a):, (b, (c),: see table (2), (d): Very highly significantly different (P<0.001).

2.Enzymatic Variation in *Adesmia* sp.:

Spatial and seasonal variation in the activities of (LDH) and (AchE). Spatially, annual mean (Table 2) in the activity of lactate dehydrogenase (LDH) recorded its maximal activity at the sites of heavy and medium industries (93.75 ± 21 & 90 ±21.2 u/mg, respectively); its increment was contributed to more activity of this enzyme during summer (184 ±1.82 & 183±1.82u/mg, respectively) and winter (146 ± 1.82 & 131 ±1.82 u/mg, respectively). Meanwhile, the least sites (66.7 ±13.3 u/mg) were at the control ones where the minimal activity of enzymes (15 ±1.82u/mg) during autumn. Moreover, an extremely significant difference along spatial and seasonal variation was in the activity of lactate dehydrogenase (LDH).

On the other hand, the maximum activity of (AchE) showed during summer (56.7 ± 1.4u/mg) and reduced during autumn and spring (17.0 ± 1.4& 16.75 ± 1.4u/mg). Among the study sites, sites of heavy industries recorded the maximal activity (96± 1.4u/mg) of AchE during summer but spring was the minimal value (9± 1.4u/mg) of its activity there. In addition, these sites achieved the highest mean (96± 1.44u/mg) in the activity of AchE, on the opposite to the activity of this enzyme at control ones (19.9 ±3.3 u/mg). Finally, the results also achieved the spreading of enzyme activity seasonally and spatially between different study seasons and sites.

Table (2). Seasonal and spatial variation in the activities of (LDH) and (AchE) in *Adesmia* sp.

Enzymes	Species	Study seasons			
		A	B	C	D
LDH	*Cataglyphis savignyi*	43.3±2.02[c]	45.35±0.79[b]	60.68±1.35[b]	78.33±2.6[a]
	Adesmia sp	66.7 ±13.3 [b]	71.75 ±16.3 [b]	90 ±21.2 [a]	93.75 ± 21[a]
	Chrotogonus homalodemus	16.01±4.4[c]	83.6 ±20.11 [b]	95.5 ± 18.3[a]	98.3 ±8.05[a]
AchE	*Cataglyphis savignyi*	25.4±0.6[c]	32.8±1.8[b]	34.3±1.9[b]	38.92±1.17[a]
	Adesmia sp	19.9 ±3.3 [c]	22 ±3.25 [c]	31.25 ±4.7 [b]	41 ±10.6 [a]
	Chrotogonus homalodemus	46.01 ±4.02 [b]	46.9 ±4.2 [b]	79.4±11.9[a]	77.9±19.7[a]

The given values are the means ± standard error. Post hoc test indicates that the different superscript letters are significantly different, Note that, A, control sites; B, sites of light industries; C, sites of medium industries; D; (a): Not significantly different (P>0.05), (b): Significantly different (P<0.05), (c): Highly significantly different (P<0.01).

3.Enzymatic Variation in *Chrotogonus homalodemu:*

The activity of antioxidant enzymes: (LDH) and (AchE) were seasonally measured at different study sites. Significant variation was detected between study sites at different study sites at *P*< 0.01&0.001 except during autumn in the activity of (AchE).

Spatially, the results reached the dispersal of enzyme activity seasonally at different study sites. No difference (Table 3) was detected between the maximal values at the sites of medium and heavy industries (LDH, 95.5-± 18.3 & 98.3 ±8.05 u/mg, respectively) in the activity of lactate dehydrogenase (LDH) and (AchE, 79.4±11.9& 77.9±19.7 u/mg, respectively) in the activity of (AchE). Meanwhile, the minimal activity of both enzymes was at control (LDH, 16.01±4.4& AchE, 46.01-±4.02 u/mg).

Spatial and seasonal variation of the activity of lactate dehydrogenase (LDH) was illustrated in Figure (3). The maximal value was at sites of heavy industries during autumn (241±2.3 u/mg) and sites of medium industries during summer (189±0.58u/mg). The minimal value of its activity was at control one during autumn and spring (1.0 ± 0.57 & 2.033 ± 0.5u/mg, respectively). On the other hand, sites of heavy and medium industries recorded the maximal value of its activity during summer (183±1.7& 144-±2.3u/mg, respectively). Meanwhile, the minimal value (27.3 ±0.33 u/mg) was at sites of heavy industries during spring.

4.Enzymatic Biomarker:

Spatially, the activity of both enzymes clarified their reponse towards increasing the heaviness of industries either in the activity of (LDH) and in (AchE) as clarified by Fig. 4& in 5. Also, in comparing between the study species in their level of enzymatic activity, The level of lactate dehydrogenase (LDH) recorded the gradual increasing (Table 2& Fig. 4) of its activity in *Cataglyphis savignyi* and *Adesmia* sp. from control (43.3±2.02 & 66.7 ±13.3 u/mg, respectively) to sites of heavy industries (78.33±2.6& 93.75 ± 21 u/mg, respectively). Meanwhile, *Chrotogonus homalodemus* was sharply increasing in the activity of this enzyme from 16.01±4.4u/mg in control to be 98.3 ±8.05u/mg at sites of heavy industries.

Figure (5) clarified the more responsive of (AchE) in *Cataglyphis savignyi* and *Adesmia* sp. towards metals accumulation by increasing the activity of this enzyme from at control sites (25.4±0.6 &19.9 ±3.3 ^{u}/mg, respectively) to at sites of light industries (32.8±1.8 &22-±3.25 ^{u}/mg, respectively) then gradual increase towards heaviness of industries. Meanwhile, no increasing in the activity of this enzyme between control and sites of light industries but sharp increasing with industries to end with 77.9±19.7 u/mg in sites of medium industries and 79.4±11.9 u/mg in sites of heavy industries.

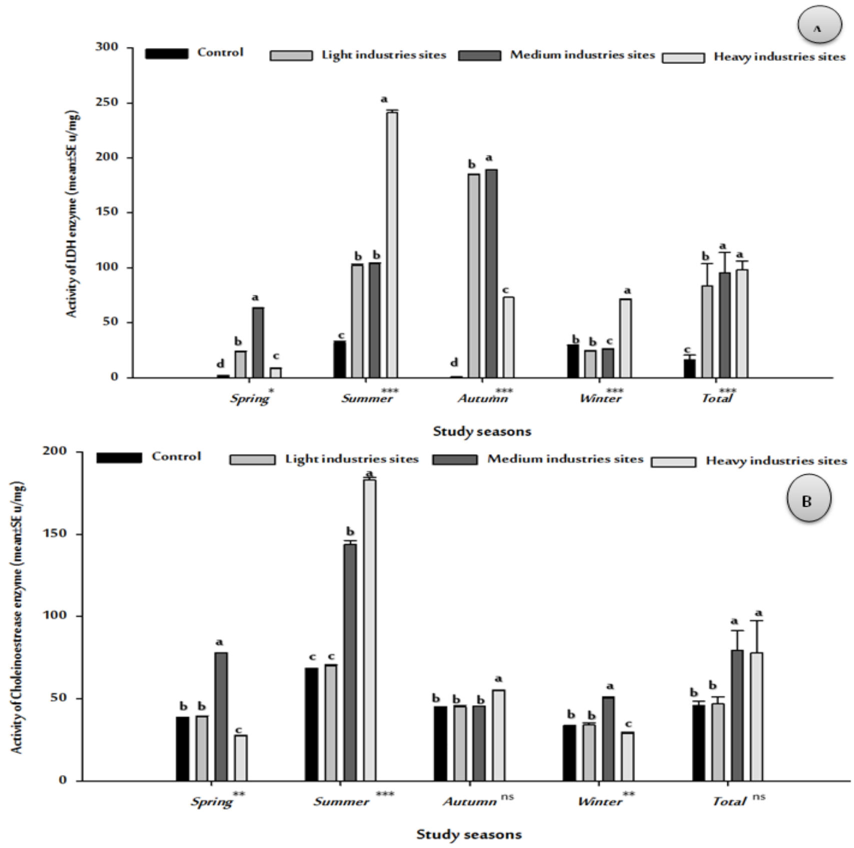


Fig. (3). Spatial and seasonal variation of the activity of LDH and AchE enzyme (mean± SE u/mg) in grasshopper, *Chrotogonus homalodemus*, at different study sites throughout study period, El-Sadat city, Menofia Governorate. Post hoc test indicates that the different superscript letters are significantly different, while the symbols [ns, *, **, and ***] :see table (1), (a):, (b, (c),: see table (2). (d) See fig (2)

Table (3). Spatial variation in the activities of (LDH) and (AchE) at different study period, El-Sadat city, Menofia Governorate.

Enzymes	Species	Study seasons			
		A	B	C	D
LDH	*Cataglyphis savignyi*	43.3±2.02^{c}	45.35±0.79^{b}	60.68±1.35^{b}	78.33±2.6^{a}
	Adesmia sp	66.7 ±13.3 ^{b}	71.75 ±16.3 ^{b}	90 ±21.2 ^{a}	93.75 ± 21 ^{a}
	Chrotogonus homalodemus	16.01±4.4^{c}	83.6 ±20.11 ^{b}	95.5 ± 18.3^{a}	98.3 ±8.05^{a}
AchE	*Cataglyphis savignyi*	25.4±0.6^{c}	32.8±1.8^{b}	34.3±1.9^{b}	38.92±1.17^{a}
	Adesmia sp	19.9 ±3.3 ^{c}	22 ±3.25 ^{c}	31.25 ±4.7 ^{b}	41 ±10.6 ^{a}
	Chrotogonus homalodemus	46.01 ±4.02 ^{b}	46.9 ±4.2 ^{b}	77.9±19.7^{a}	79.4±11.9^{a}

Note that; A,; B,; C,; D; (a):, (b, (c),: see table (2).

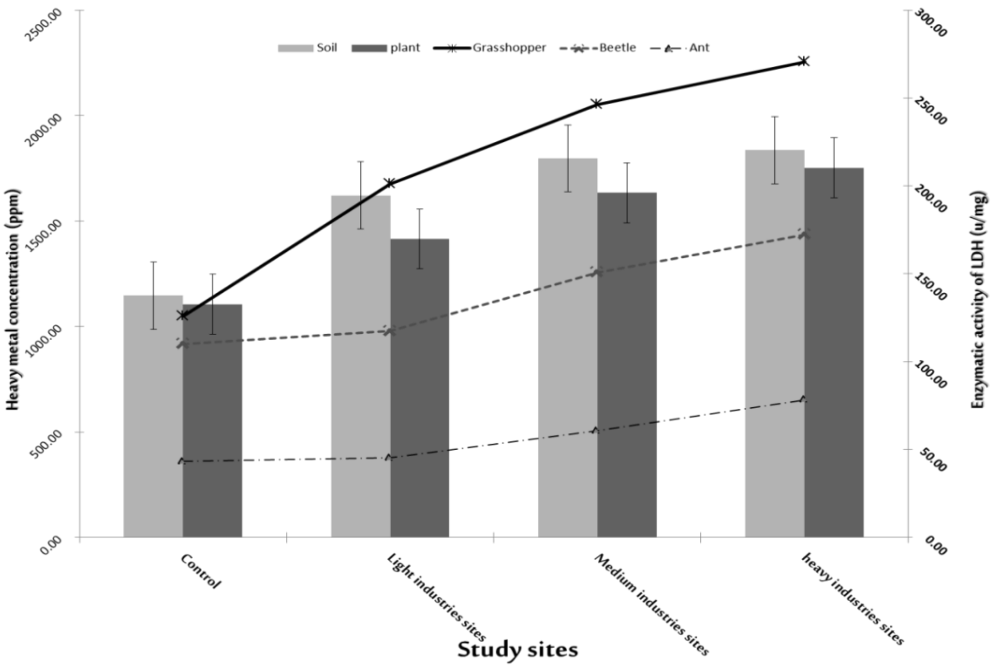


Fig. (4). Enzymatic biomarker of lactate dehydrogenase (LDH) for heavy metals accumulation in soil and flora at study species at different study sites in El-Sadat industrial city, Menofia Governorate, Egypt. Note that, ant, *Cataglyphis savignyi*; beetle, *Adesmia* sp.; and *Chrotogonus homalodemus*.

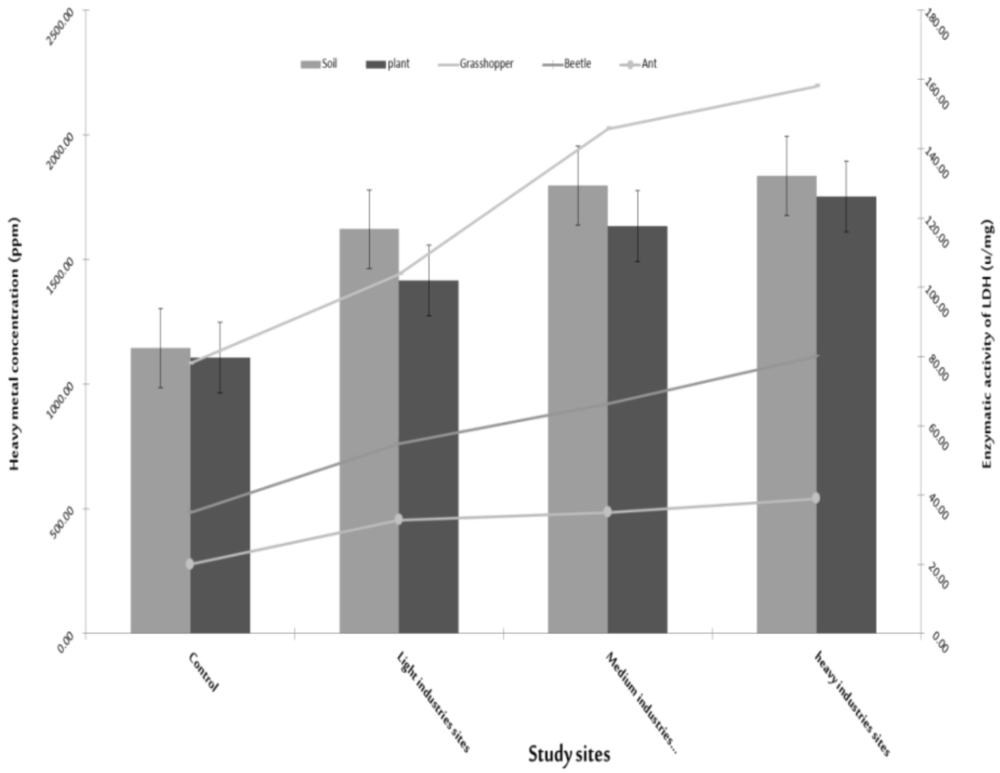


Fig. (5). Enzymatic biomarker of (AchE) for heavy metals accumulation in soil and flora at study species at different study sites in El-Sadat industrial city, Menofia Governorate, Egypt. Note that, ant, *Cataglyphis savignyi*; beetle, *Adesmia* sp.; and, *Chrotogonus homalodemus*.

Response of Enzymatic Biomarker to Bioaccumulation of Heavy Metals:

Pearson's correlation coefficient (r) detected the response of both enzymatic biomarker towards the different industrial degree and bioaccumulation of different heavy metals throughout different relationships, as shown in Table (4). A positive correlation was attained between both enzymatic biomarkers: (LDH) and (AchE) at r=0.4, p<0.01 throughout the response of different study species towards heavy metals' bioaccumulation. In special, the accumulation of copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) were positively correlated with the concentration of (LDH) and (AchE) at body fluid of different study species. The more positive correlated of lactate dehydrogenase (LDH) was clarified towards the bioaccumulation of zinc (Zn: r=0.518) < manganese (Mn: r= 0.510) < and copper (Cu: r=0.416) at p<0.01. While, (AchE) was more responded towards iron bioaccumulation (r= 0.511 at p<0.01). Finally, it is not strange to detection the positive correlation between the ordering of study sites according to industries and both enzymatic biomarkers (LDH, r= 0.57 at p<0.05; and AchE, r= 0.57 at p<0.01).

Table (4). Pearson's correlation coefficient (r) between different heavy metals' bioaccumulation, enzymatic biomarker (LDH and AchE) at different study species, and different degree of industries throughout study in El-Sadat city, Menofia Governorate.

	Degree of industries sites	Heavy metals at different study species			
		Fe	Zn	Mn	Cu
Degree of industries sites	1	0.066	0.334**	0.365**	0.373**
lactate dehydrogenase (LDH)	0.743**	0.466**	0.518**	0.510**	0.416**
Acetylcholinesterase (AchE)	0.57*	0.511**	0.352**	0.18	0.280*
Zinc (Zn)	0.334**	0.694**	1	0.749**	0.321**
Copper (Cu)	0.373**	0.501**	0.321**	0.358**	1
Iron (Fe)	0.066	1	0.694**	0.615**	0.501**
Maganium (Mn)	0.365**	0.615**	0.749**	1	0.358**

Note that; * and **: see table (1).

DISCUSSION

Accumulation of Heavy Metals in Soil and Plant:

Soil is considered powerful tracers for monitoring impact of anthropogenic activity especially industrial emission in addition to plants which are direct uptake heavy metals from soil and surrounding environment by their roots, stems or shoot (Monni *et al.*, 2001; Lu *et al.*, 2012; Soriano *et al.*, 2012). Therefore, the accumulation of heavy metals in soil and flora was detected in the current study sites.

The current analysis of heavy metals in soil detected their accumulation order from high to low concentrations as follows: Fe > Mn > Zn > Cu > Cd. While in flora as follows: Fe > Zn > Mn > Cu > Cd. Where, increasing the content of heavy metals was at sites of heavy (site D) and medium industries (site C) than elsewhere, especially for D in soil and C in flora. This result attributed to the increasing the emission of heavy metals at heavy and medium industries more than other, the results of Mustafa and El-Shazly (2017) who stated the increasing of metals with the nearness of industrial pollution sources.

Also, our results indicated that iron is the highest trace metal because this element characterizes by its surfactant and correlation properties and stabilizes trace metals by complexion and also includes particulate matter as was stated by different authors (Rashid and Leonard, 1973; Harrison and De Mora, 1996).

On the other hand, the concentration level of Mn detected in this study increased followed by iron which increasing using of different heavy and medium industries and releasing with domestic and industrial waste as revealed by Rani and Reddy (2003) ; Khaled (2004) and Osman (2012) in their researches when testified the enrichment of this element due to the domestic and industrial waste. Meanwhile, increase Zn due to industrial effluent and this agreement with An (2004) and Cui *et al.* (2004) who reported that soil and sediment was heavily polluted with a mixture of metals from industrial effluent and this ultimately affects the physiological parameters of organisms in the soil ecosystem, some of the examples include: cadmium (Cd) and zinc (Zn).

Finally, in the soil and plant subsystem, the most concentration of heavy metals was higher in soil, especially at a heavily industrial area. This fact was accordant with the detection of the current soil analysis which had been polluted by heavy metals and was in agreement with the observation of (Zhang *et al*., 2009).

Enzymatic Biomarker:

Organism uses various mechanisms to deal with additional stress caused by heavy metals accumulation in their body. They either store metals in different organs of their body (Ludwig and Alberti, 1988) or eliminate them from their bodies via excretion or molting (Lee *et al*., 1978). Moreover, the use of biochemical biomarkers to measure the exposure of organisms to chemical pollution and environmental contamination has been extensively reviewed (Augustyniak *et al*., 2005; Min and Kang, 2008; Wilczek *et al*., 2008; Praet *et al*., 2014). They also exhibit a variety of enzymatic activities to metabolise or degrade different xenobiotics (Augustyniak *et al*., 2005;Wilczek *et al*., 2008). In addition, enzymes protect cells from oxidative damage that inhibits antioxidant activity thus secure more important molecule like DNA, RNA and proteins (Augustyniak and Migula, 2000).

On our results, the activity AChE was found higher in heavy and medium industrial sites may be due to heavy metals enhancement of acetylcholine at the synapses, so that the post-synaptic membrane is in a state of permanent stimulation.

(AChE) plays an important role in neurotransmission in both vertebrates and invertebrates, being responsible for the hydrolysis of acetylcholine into choline and acetic acid at the cholinergic synapses and neuromuscular junctions (Peña-Llopis *et al.*, 2003)**.**

This agreed with Gill *et al.* (1991), who reported increased AChE activity in skeletal muscles and brain of a fish species exposed to Cd for 48 hours. Also, Zatta *et al.* (2002) observed increases in AChE activity of rats treated orally with Alumine. Also, Sun *et al.* (2008) observed that increase in AChE activity with the increase of xenobiotics. Moreover, Qin *et al.* (2012) investigated that spiders of highly polluted areas may have developed some compensatory mechanisms which cause increase synthesis of enzyme molecule and high AChE activity. On the opposite facet, Lavado *et al.* (2006) reported that AChE was strongly inhibited in the muscle of some invertebrate sampled from polluted river by organophosphorous, carbamates, and heavy metals. Furthermore, activity inhibition was correlated with Zn, Cu and Cd accumulation in digestive gland and gills of treated clams (Kamel *et al*., 2012)**.** Butt and Aziz (2016) clarified that under high bioaccumulation of heavy metals of Cr and Cu leads to decrease of detoxifying enzymes.

Ketterer *et al.* (1983) represented the clarifying explanation, which a positive relationship indicates that under a higher metal load index GST levels are upregulated thereby preventing oxidative damage by converting breakdown products of lipid peroxides to glutathione.

Barata *et al.* (2005) analogously observed positive (Fe and Pb) or no (Cd and Cr) correlations between accumulated individual metal concentrations and several antioxidant enzymes (CAT and GST) for comparable body burdens in Trichoptera larvae (*Hydropsyche exocellata*), except for Cd. Meanwhile, Ketterer *et al.* (1983) represented the clarifying explanation which a positive relationship indicates that under a higher metal load index GST levels are up there by preventing oxidative damage by converting breakdown products of lipid peroxides to glutathione.

On the other hand, LDH is an important glycolytic enzyme involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantino *et al.*, 2001). Our results showed that increase activity of LDH in heavy and medium industrial sites and this disagreement with other researchers who observed in different insects exposed to different insecticidal stress. In *Culex sp*. after treatment with DDT, Malathion and cyfluthrin, LDH decreased with highly dramatic ratios (Arshad *et al.*, 2002). Also, Nathan *et al.* (2005) showed that treatment of *S. littura* with azadirachtin highly decreased this enzyme in the midgut. Similar results also were achieved in case of the rice striped stem borer treated with diazinon (Zibaee *et al.*, 2008).

Finally, our results suggested that we can be used AChE a bioindicator for heavy metals in collected insects.

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ARABIC SUMMARY

تقييم التلوث الصناعي من خلال المؤشرات الحيوية الأنزيمية في أماكن مختلفة من مدينة السادات الصناعية، المنوفية، مصر.

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التلوث الصناعي هو أحد أكثر التلوثات خطورة والتي تشكل تهديدا مستمرا للبشرية. يعد تقييم التلوث الصناعي هو الهدف الرئيسي للدراسة الحالية من خلال الكشف عن تراكم المعادن الثقيلة في الموائل المحيطة بها وخاصة التربة والنباتات وكذلك استجابة الحشرات تجاه هذا التلوث. لذلك، تم إجراء جمع موسمي لثلاثة أنواع مختلفة من الحشرات بالإضافة إلى أخذ عينات من التربة وأنواع النباتات الشائعة، في مواقع مختلفة في مدينة السادات الصناعية، المنوفية، مصر لمدة أربعة مواسم متتالية خلال عام 2016 إلى عام 2017. تم استخدام انزيم أسيتيل كولين استيراز (AChE) وانزيم لاكتات دي هيدروجيناز (LDH) المستخلص من كامل الجسم المتجانس من الحشرات المختارة كمؤشرات حيوية للتلوث المعادن الثقيلة. أيضا، تم تحليل عينات النبات والتربة لمحتويات المعادن الثقيلة. أوضحت النتائج العديد من الأنشطة التي قام بها المرقمان الحيويان في مواقع الدراسة المختلفة، حيث قدمت صورة تمثيلية للحالة البيئية للمناطق الصناعية التي تمت دراستها، كما تم اختبار أنواع مختارة من الحشرات كعلامات بيوكيميائية حيوية وأداة سريعة الاستجابة تجاه هذا التلوث.