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Tracing industrial pollution: unveiling environmental health via insects' biomarkers

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Abstract

The objective of this work was to analyze the efficacy of *Cataglyphis saviginyi* and *Tentyrum Sp* enzymatic biomarkers in evaluating environmental pollution in industrial areas of Borg El-Arab City, Egypt. The collection of *C. saviginyi* and *Tentyrum Sp* seasonal specimens for four consecutive seasons in 2020 and 2021, together with soil samples from the site locations, was conducted to quantify heavy metal loadings. An analysis was conducted on the lipids, proteins, carbohydrates, and enzymatic activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) in the examined specimens. Metal contamination indices for zinc, chromium, cadmium, and copper were calculated. The analysis revealed notable variations in soil pollution levels between the control site and Industrial Sites 1 and 3. Specifically, the control site had the highest level of Zn contamination, while Industrial Sites 1 and 3 had the lowest. Similarly, the control site had the highest level of Cd contamination and the lowest level of Cu contamination. The growing industrial operations in the chemical and silicate sectors in the examined regions led to significantly elevated pollution levels at industrial site 3. A decrease in heavy metal concentration resulted in an increase in the biological accumulation factor (BAF). Compared to the control site, the industrial site exhibited elevated GPT, GOT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACPh), and protein activities, while Lactate Dehydrogenase (LDH), lipid, and carbohydrate levels were reduced. *Tentyrum Sp* exhibited increased ALP and lipid levels compared to the control site, but showed a decreasing trend in GPT, GOT, ACPh, LDH, protein, and carbs. The findings of this study offer evidence in favor of using insect bioindicators as effective instruments for identifying and tracking environmental contamination. Promising biomarkers include antioxidant enzyme activity and key metabolites.

Keywords *Cataglyphis saviginyi*, *Tentyrum Sp*, Enzymatic activity, Metabolic responses, Industrial areas

1 Introduction

From a scientific perspective, environmental contamination remains a critical issue that persists across the globe. This topic has garnered significant attention due to the widespread changes in ecosystems, primarily driven by human activities. The release of unprecedented amounts of chemical substances into the environment is disrupting the natural processes of ecosystems. Chemization not only affects plants and animals but also has a notable impact on non-living components of the environment [1]. Moreover, industrial pollution serves as a major contributor to environmental change, with far-reaching effects on both aquatic and terrestrial habitats, profoundly influencing animal populations [2].

Biomarkers may be utilized to offer early warning signals for several contaminants, as stated by Depledge [3]. Researchers have utilized a wide range of biomarkers as indicators of ecological risk in ecotoxicological monitoring studies [4]. The human causes of climate change and its consequences for population growth are still being studied in depth [5]. Insects' ecology, physiology, growth, development, genetics, and behavior have all been significantly influenced by heavy metals (HMs) [6].

When it comes to maintaining a healthy terrestrial ecology, insects play a key role [7]. Insects are extensively

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utilized, rendering them exemplary models for examining environmental alterations and assessing the impacts of HM pollution [8]. Environmental HMs are ingested by insects and eventually accumulate in their digestive systems [9]. When compared to other invertebrate species, ants have a higher tolerance for HM contamination [10]. On the other hand, pollution has been shown to have negative effects on ant biodiversity [11], abundance, community structure [12], and colony size [13]. Pollution also causes smaller workers to be more common in certain ant species [13]. Research on metal accumulation in ants for bioindication should acknowledge their status as social insects. In ants, fundamental lower-level units amalgamate to construct intricate structures, notably the colonies, which represent a unique organizational tier. Comprehensive understanding of metal accumulation mechanisms in ants, and consequently its application for bioindication, necessitates studies at this organizational level [14, 15]. Due to habitat variety that is characteristic of all biogeographic areas and to the ally-induced landscape transformation, the coleopteran family (Tenebrionidae) is regarded as an excellent bio-indicator for soil pollution [14].

Detoxification enzymes react to various xenobiotics and HMs through metabolic routes, degradation pathways, and antioxidative functions to safeguard essential molecules and organs [16]. The activity of detoxifying enzymes is a commonly utilized, rapid, accurate, and sensitive biochemical indicator for monitoring environmental pollution [17, 18].

The major purpose of this research was to examine the prevalence of HMs in the soil of Borg El-Arab City, Egypt, to learn more about the effects of industrial pollution on the local ecosystem. The research also intends to evaluate the efficiency of pollution indicators for thoroughly assessing the level of soil contamination with HMs. In addition, the study aimed to assess the antioxidant parameters lactate dehydrogenase (LDH), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), and acid phosphatase (ACPh) for their potential as indicators of HM exposure in *Cataglyphis saviginyi*, *Tentyrum Sp.*, and their metabolites enzymes.

2 Materials and methods

2.1 Study area

The Mediterranean Sea forms the northern boundary of the Borg El-Arab area. Land use shifts have made the investigation area a prime target for a wide range of alteration types [19]. The city has nine dwelling sections and 4 manufacturing zones. There were 510 factories from various industries within the 4 industrial zones [20]. Sites were chosen to reflect both high- and low-contamination zones, ensuring diverse environmental conditions for comparative analysis. Seasonal surveys of ground fauna were done at several industrial sites and one control site (placed at least 4 km from the closest human activity), four subsequent seasons; summer, 2020 to spring, 2021. Using a portable Global Positioning System, we recorded the coordinates of each location, alongside soil samples from each site. This approach provided temporal insights into seasonal variations in both insect enzyme activities and HM accumulation in soil. Insect specimens were collected using pitfall traps placed systematically across the selected sites. These traps were left open for 48 h per sampling event. Collected insects were carefully preserved and transported to the laboratory for further biochemical and enzymatic analyses (Fig. S1).

2.1.1 Cont.

Natural environment in a desert area, located at N 30°52′58.2"—E 29°35′40.2," not far from the New Borg Al-Arab Bus Stop on the route between Cairo and Alexandria.

2.1.2 Ind. (1)

Located in the 1st Industrial Zone of Bahig, Borg Al Arab Al Gadida City, Alexandria Governorate (coordinates: N 30°52′37.4", E 29°36′54.3"). El-Amria for Rubber & Plastic Industry (Faroplast), Centra for Medical Supplies, EL-Tawheed for Advanced Industries (Sparkle shampoo and conditioner), and Fredex Firefighting & Metal Products are just some of the factories that make up this community's web presence.

2.1.3 Ind. (2)

Located at a coordinate of N 30°52′19.9" and E 29°37′07.9" in the 2nd Industrial Zone of Bahig in Borg Al Arab Al Gadida City in the Governorate of Alexandria. Various sorts of manufacturing facilities, including the Sika—Borg El-Arab Factory, the Macris Silicates Factory, the Elola Steel Group, and the Arabian Mills Company, are all shown here.

2.1.4 Ind. (3)

Located in the 2nd Industrial Zone of Bahig, Borg Al Arab Al Gadida City, Alexandria Governorate (coordinates: N 30°52′17.6", E 29°37′27.7"). Targo Chem for chemical industries, Borg Al Arab Industries- Abaza, National Co. For Oil & Grease Pentra paints firm, ACC Construction Chemicals, and Al Asdeqa Dairy Factory are all located in this area, as are many more.

2.2 HM analysis

Soil samples were collected from a depth of 0–10 cm at each site. A composite sampling approach was employed, where soil from five random points at each site was pooled to ensure a representative sample. The samples were first dried, sieved, and analyzed to determine the heavy metal content of the soil. The soil was digested using the Perchloric Acid Digestion method as described by Hesse [21]. Heavy metals in the insects were quantified using [Varian Vista AX CCD Simultaneous Inductively Coupled Plasma Atomic Emission Spectroscopy] (ICP-AES), with results expressed in parts per million (ppm) based on dry weight, following the procedures of Bream et al. [18] and the USEPA [22]. Additionally, a blank solution was prepared to ensure that neither the acid nor the distilled water used for sample dilution contained detectable traces of metals.

2.3 Biochemical analysis of enzymes

Biochemical biomarkers are more sensitive and represent early observable reactions to environmental changes, hence they are crucial for detecting the exposure of organisms to chemicals and environmental pollution [17, 23, 24]. The assays were conducted at 37 °C with a 30-min incubation time. Specific reagents were used for each enzyme assay, including α-ketoglutarate, L-alanine, and dinitrophenylhydrazine for GPT and GOT; p-nitrophenyl phosphate for ALP and ACPh; and pyruvate and NADH for LDH. The main metabolites (proteins, lipids, and carbohydrates) were measured using established methods [5]. All assays were performed in optimized buffer solutions, with spectrophotometric measurements and negative controls included. GPT, GOT, ALP, ACPh, LDH, and the main metabolites (protein, lipid, carbohydrate) were identified as promising enzymes for usage as biomarkers in this investigation [7, 25–29].

2.4 Pollution indices

The level of pollution was determined by using a number of different metrics.

2.4.1 Contamination factor (CF)

Soil contamination was evaluated using the CF and soil contamination degree, based on the method proposed by Hakanson [30]. The comparison was made between the current concentrations and the pre-industrial baseline levels, which were determined from previously published studies.

$$CF = \frac{\text{Msample}}{\text{Mbackground}}$$

where Msample and Mbackground are HM concentrations of the examined soil sample and the background, respectively. As, Cd, Cr, Cu, Pb, Zn, Ni, and Hg were measured at 13.4, 1, 69, 39, 17, 67, 55, and 0.08 ppm, respectively, in the background [31]. According to Hakanson [30], the contamination levels range from 0 to 3 for low contamination to 6 for severe contamination (very high contamination).

2.4.2 Degree of contamination (CD)

Hakanson [30] proposed the CD as a tool for assessing pollution by summing the CF of several metals. However, it is important to note that the CD value can increase with the number of metals measured, potentially leading to higher values even in cases where individual contamination levels are low. To account for this, the Modified Degree of Contamination (mCd), which averages the CF values across all elements, offers a more balanced approach. The classification remains: CD < 6 (low contamination), 6 < CD < 12 (moderate contamination), 12 < CD < 24 (considerable contamination), and CD > 24 (high contamination).

$$\text{Cdeg} = \sum_{i=1}^{n} \text{CFi}$$

For every element, CFi is the contamination factor. Soil contamination is measured on a scale from 0 to 32 on the Cdeg scale, with 0 indicating the least contaminated, 8 indicating moderate contamination, 16 indicating substantial contamination, and 32 and higher indicating very high contamination [30, 32].

2.4.3 Modified Cd (mCd)

According to Abrahim and Parker [33], Machender et al. [34], and Rahman et al. [35], at least three chemical elements (e.g., cadmium, chromium, and copper) must be employed in order to calculate the modified degree of contamination (mCd), which is the average value of pollution indices for all trace elements (CFi).

The soil classified into seven-order scale of contamination, which is: very low contamination ($m\text{Cdeg} \leq 1.5$), Low contamination ($1.5 \leq m\text{Cdeg} \leq 2$), Moderate contamination ($2 \leq m\text{Cdeg} \leq 4$), High contamination ($4 \leq m\text{Cdeg} \leq 8$), Very high contamination ($8 \leq m\text{Cdeg} \leq 16$), Extremely high contamination ($16 \leq m\text{Cdeg} \leq 32$), Ultra-high contamination ($32 \leq m\text{Cdeg}$). This index is calculated using the following equation:

$$\text{mCdeg} = \frac{1}{n} \sum_{i=1}^{n} \text{CFi}$$

where n is the number of elements evaluated.

2.4.4 Geo-accumulation Index (Igeo)

The Igeo has been used as a geochemical criterion to assess the level of contamination of a particular element in sediments or soils in the environment since 1969. The following is the equation used to derive Igeo values. Buhari and Ismai [36]:

$$Igeo = \log_2\left(\frac{Cn}{1.5 * Bn}\right)$$

where Cn represents the concentration of the measured element in the soil and Bn is the geochemical background value of the provided metal. The number 1.5 is a correction factor for the background matrix that takes into account the impacts of lithogenic fluctuations on natural fluctuations. As, Cd, Cr, Cu, Pb, Zn, Ni, and Hg were all measured as having background concentrations of 13.4, 1.69, 39.2, 55.7, and 0.08 mg L^{-1}, respectively, in this investigation [31].

The Igeo is divided into seven levels: Igeo ≤ 0, uncontaminated; 0 < Igeo ≤ 1, uncontaminated to moderately contaminated; 1 < Igeo ≤ 2, moderately contaminated; 2 < Igeo ≤ 3, moderately to heavily contaminated; 3 < Igeo ≤ 4, heavily contaminated; 4 < Igeo ≤ 5, heavily contaminated to extremely contaminated; and 5 ≤ Igeo, extremely contaminated [37–39].

2.4.5 Pollution load index (PLI)

A PLI is a tool that has been used to evaluate the quality of soil. The equation is calculated by taking the square root of the product of the contamination factors of the items of interest.

$$\mathrm{PLI} = (\mathrm{CF}_1 \times \mathrm{CF}_2 \cdots\cdots \mathrm{CFn})^{1/n}$$

where, CF_1, CF_2, and CF_n are the contamination factors of the elements 1, 2, and n. This index classifies the soil into three categories, which are: Polluted (PLI > 1), Baseline levels of pollution (PLI = 1) and, Not polluted (PLI < 1).

2.4.6 Pollution index (PI)

Calculating the contamination level of particular elements requires first defining the PI, which is the ratio of the HM concentration to the geometric mean of background concentrations [37, 40, 41]. The calculation of PI is as follows:

$$PI = \sqrt{\frac{(CFaverage)2 + (CFmax)2}{2}}$$

where the highest contamination factor (CFmax) and the average contamination factor (CFaverage) are given. Low contamination (PI ≤ 1.0), moderate contamination (1.0 < PI ≤ 3.0), and high contamination (PI > 3.0) are determined using the PI value of each metal at each sample location [39, 40].

2.4.7 Bioaccumulation factor (BAF)

Insect HMs accumulation was compared to soil HMs accumulation (μg g^{-1} dry weight) from the same locations using the BAF. Insect samples were categorized using the BAF as either deconcentrates (BAF < 1), which release the metal in the soil, micro concentrators (1 < BAF < 2), or macro concentrators (BAF > 2). According to Van Gestel et al. [42], the BAF of tested HMs in the beetle samples was calculated as follows:

$$BAF = \frac{HMinsect}{HMsoil}$$

where HMinsect = HMs accumulation in the body of Insect (μg g^{-1} tissue), and HMsoil = HMs in soil (μg g^{-1}).

2.5 Statistical analysis

SPSS V.22 was utilized for data coding and entry as a statistical program. Parametric test assumptions were checked using the Shapiro-Wilk and Kolmogorov-Smirnov tests for normally distributed continuous data. To ensure the normality of the probability and percentile data, we used the Arcsine Square Root transformation. The information was shown as a mean and standard deviation. ANOVA analyses were done for the studied locations regarding the recorded variables; analysis was evaluated using three replicates at least for each group; post-hoc analysis was checked using Tukey pairwise comparison using Mini Tab V 14; *P*-value were regarded significant at < 0.05. Regression was done to calculate the link and the prediction equation between soil HMs and *C. savignyi* Detoxification enzyme, the analysis became accessible utilizing Sigma Plot V 14.0. Using Past V 4.12, we see examples of distance clusters and Canonical Correspondence Analysis (CCA). When it was feasible to do so, data was displayed in R studio V 2022.02.4.

3 Results

3.1 HM in soil

Cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) levels in soil were evaluated at several industrial locations in Borg El-Arab (Table 1 and Fig. S2). There were statistically significant differences ($P < 0.05$) between industrial 1 and 3, and the control site, in the concentrations of Cd, Cr, and Cu. All industrial locations had significantly higher soil concentrations of Zn than the control site ($P < 0.05$).

Table 1 Heavy metal concentrations in soil samples obtained from several industrial sites in Borg El-Arab, Egypt, and their spatial distribution (in ppm)

Area	Cd (ppm)	Cr (ppm)	Cu (ppm)	Zn (ppm)
Cont	1.25 ± 0.05^{c}	27 ± 1.41^{b}	1.45 ± 0.07^{b}	351.5 ± 14.84^{b}
Ind. 1	1.55 ± 0.09^{b}	14.5 ± 3.53^{c}	1.15 ± 0.06^{c}	298 ± 5.65^{c}
Ind. 2	1.45 ± 0.07^{c}	23.5 ± 3.4^{b}	1.45 ± 0.05^{b}	228.5 ± 4.94^{d}
Ind. 3	4.5 ± 0.14^{a}	44.5 ± 5.2^{a}	1.75 ± 0.07^{a}	1468.5 ± 16^{a}

[*] Means in the column that shares the same letters is not statistically significant ($P > 0.05$)

3.2 Accumulation of HM in *C. saviginyi*

C. saviginyi at industrial 3 significantly differed ($P < 0.05$) with *C. saviginyi* at control site regarding Cr, while at industrial 1 and 2 did not differ significantly ($P > 0.05$) with the control site. *C. saviginyi* at industrial 2 was significant ($P < 0.05$) regarding Cd, Cu, and Zn with the control site. While *C. saviginyi* at industrial 1 and 3 were insignificant ($P > 0.05$) with the control site. Regarding the spatial variation, all the HM concentration in *C. saviginyi* at different industrial areas were higher than in the collected specimen at control or site. On the other hand, for seasonal variation all HMs in colder seasons were higher than hotter seasons, except Zn was high concentration in hotter seasons (Fig. 1).

3.3 Accumulation of HM in *Tentyrum Sp*

Tentyrum Sp at industrial 2 and 3 were significant ($P < 0.05$) with its concentration in *Tentyrum Sp* at control regard to Cu concentration. *Tentyrum Sp* at industrial 2 varied significantly ($P < 0.05$) with the control site for Cr concentration. While the Cr concentration at industrial 1 and 3 was insignificant ($P > 0.05$) with the control site. All industrial sites were significant ($P < 0.05$), with the control site regarding the Cd and Zn concentration in *Tentyrum sp*. Regarding the spatial variation, all the HMs in the different industrial areas were higher than the control site. On the other hand, for seasonal variation all HMs in hotter seasons were higher than colder seasons, except Cu was high concentration in colder seasons (Fig. 1).

3.4 Pollution indices

3.4.1 CF

The CF for Cd is highest in the third industrial sector, and it is moderate in the first two sectors and the control sites. In terms of Cr and Cu, contamination is negligible everywhere. Finally, Zn contamination is highest at the industrial 3 level, but still present at the industrial 1, 2, and control levels.

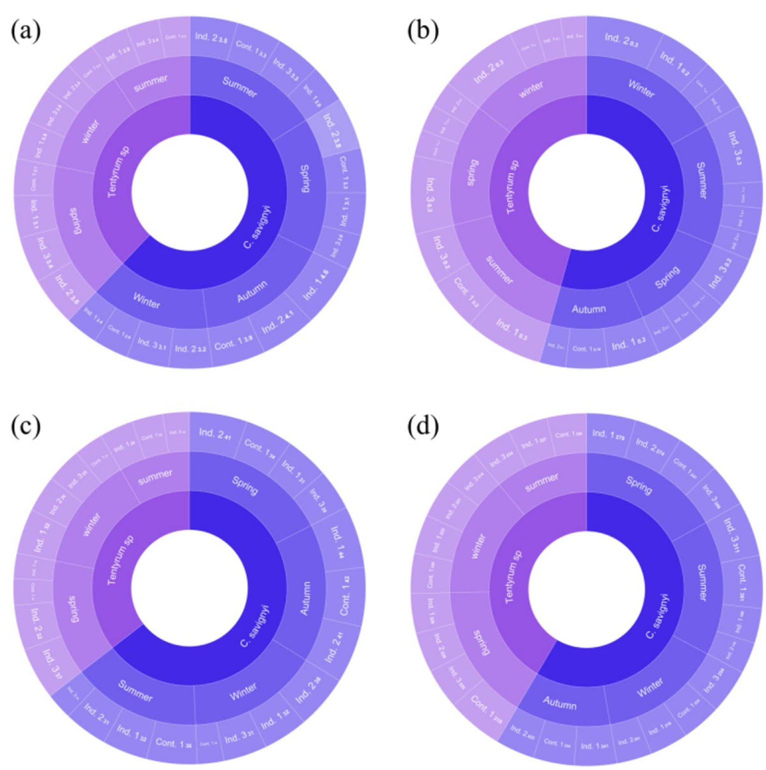


Fig. 1 Seasonal and spatial variation of heavy metals in both investigated species: (**a**) Cd, (**b**) Cr, (**c**) Cu, and (**d**) Zn

Ecological risk factor (E_r) HM pollution in soils was evaluated effectively using the risk factor. There is little to no ecological risk from Cd and Cu at all of the locations tested. In terms of Cr, all locations pose little to no ecological risk, with the exception of industrial site #3, which posed a moderate risk. When it comes to Zn, industrial 3 and the control group both posed a significant threat to the environment, whereas only industrial 1 and 2 showed any significant ecological risk (Fig. 2).

Igeo The Cd pollution levels in Industrial Zone 3 were moderate, whereas those in Industrial Zones 1, 2, and the control zone ranged from low to moderate. All locations lacked Cu contamination. Except for industrial 1, the other sites ranged from having no Cr pollution to having high Cr pollution. Finally, Zn pollution was moderate at all locations (Fig. 2).

Cdeg There was a high level of pollution in Industrial Zone 3, whereas the other two Zones and the Control Zone had far lower levels of contamination (Fig. 3).

mCd There is a high level of pollution in Industrial 3, a moderate level in Industrial 1, and a very low level of contamination in Industrial 2 (Fig. 3).

PLI Sediment deterioration due to HM buildup may be easily shown via the use of the PLI. According to PLI all sites were unpolluted, except industrial 3 was moderately polluted to unpolluted (Fig. 3).

PI According to PI indices industrial 3 was at heavy pollution, meanwhile industrial 1 and control were moderate pollution, but industrial 2 exhibits a slight pollution (Fig. 3).

3.4.2 BAF

The BAF from the locations analyzed represented the comparison between the number of HMs found in insect bodies and the amount found in the soil (g g1 dry weight) (Fig. 4).

BAF indices for C. savignyi For Cd showed that *C. savignyi* had macro-concentrator status at all sites, except in industrial 3, it was de-concentrator and released the metals into soil. Regarding Cr and Zn at all studied sites, the collected specimens from *C. savignyi* show a de-concentrator status. While for Cu macro-concentrator status was observed at all sites (Fig. 4).

BAF for Tentyrum sp. For Cd at industrial 1 and control site showed a micro-concentrator status for *Tentyrum sp*, while at industrial 2 was macro-concentrator and de-concentrator with regarded industrial 3. For Cr and Zn at all sites, the collected specimens of *Tentyrum sp* represent de-concentrator status. Finally, for Cu macro-concentrator status observed at all sites (Fig. 4).

Enzymatic biomarker in C. savignyi Seasonal variation in the activities of the enzyme as GPT, GOT, ALP, ACPh, LDH and main metabolites (Lipid, Protein,

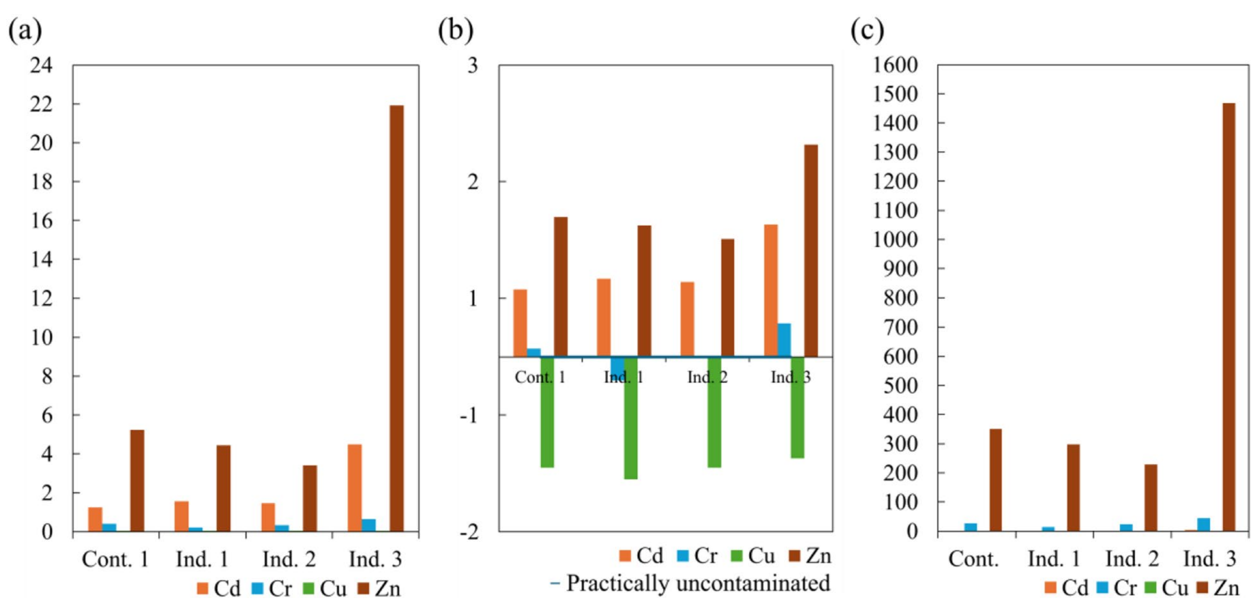


Fig. 2 **a** Cfi, (**b**) Igeo, and (**c**) Er calculated for heavy metal contamination at observed sites

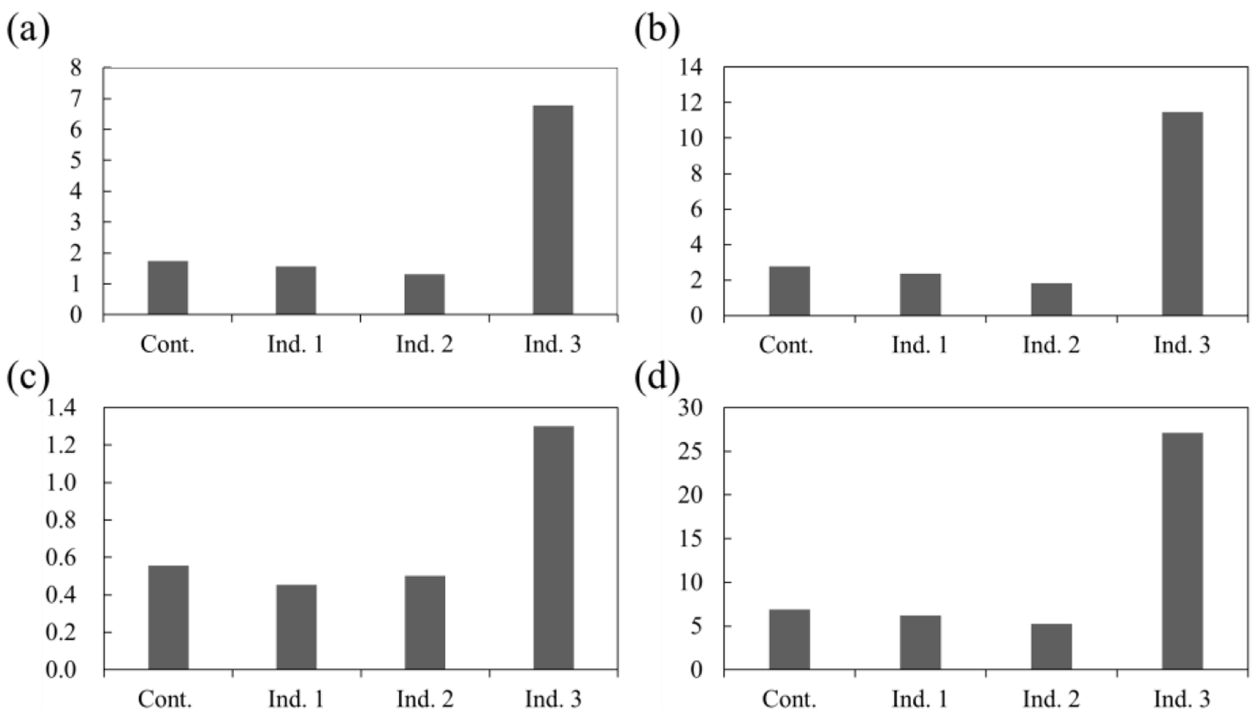


Fig. 3 **a** mCd, (**b**) PI, (**d**) PLI and (**d**) Cdeg calculated for heavy metal contamination at observed sites

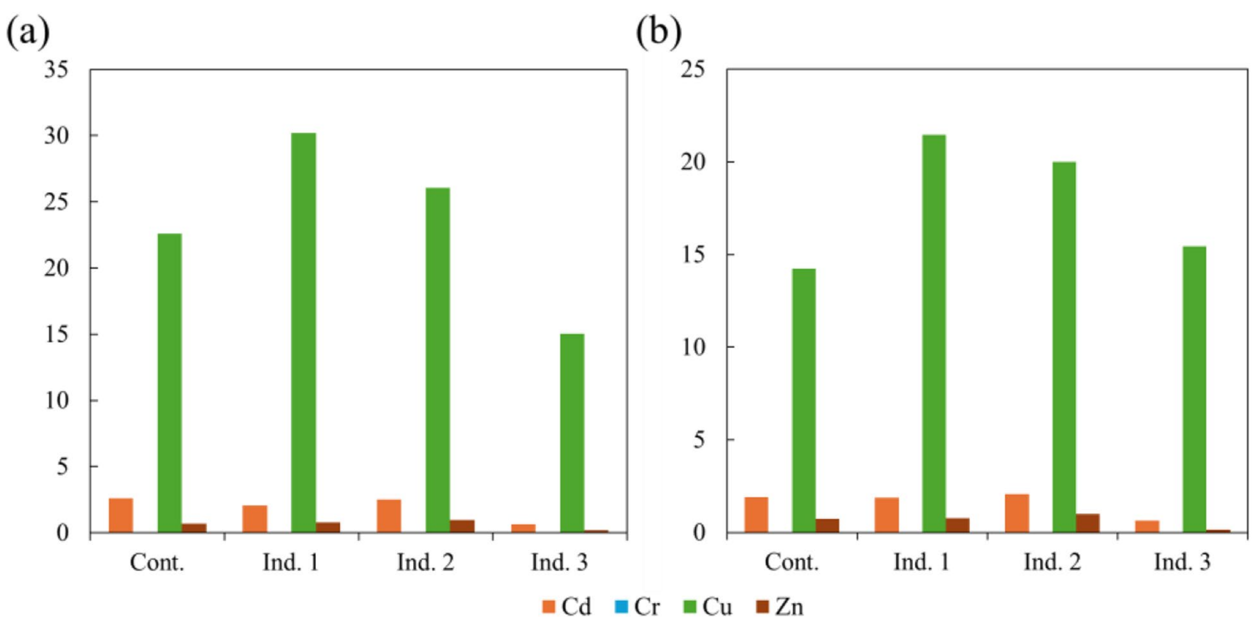


Fig. 4 Calculated Bioaccumulation factor (BAF) for (**a**) *C. saviginyi* and (**b**) *Tentyrum sp*

Carbohydrate) were detected in *C. savignyi* at Borg El-Arab (Fig. 5).

Controlling for previously observed GPT values, concerns about Industrial Areas 1 and 3 were statistically significant ($P < 0.05$). When compared to the control site, only Industrial 1's GOT values were statistically significant ($P < 0.05$), while those of Industrials 2 and 3 were not ($P > 0.05$). Comparing ALP levels among the control site and the first and third industrial sites, the former

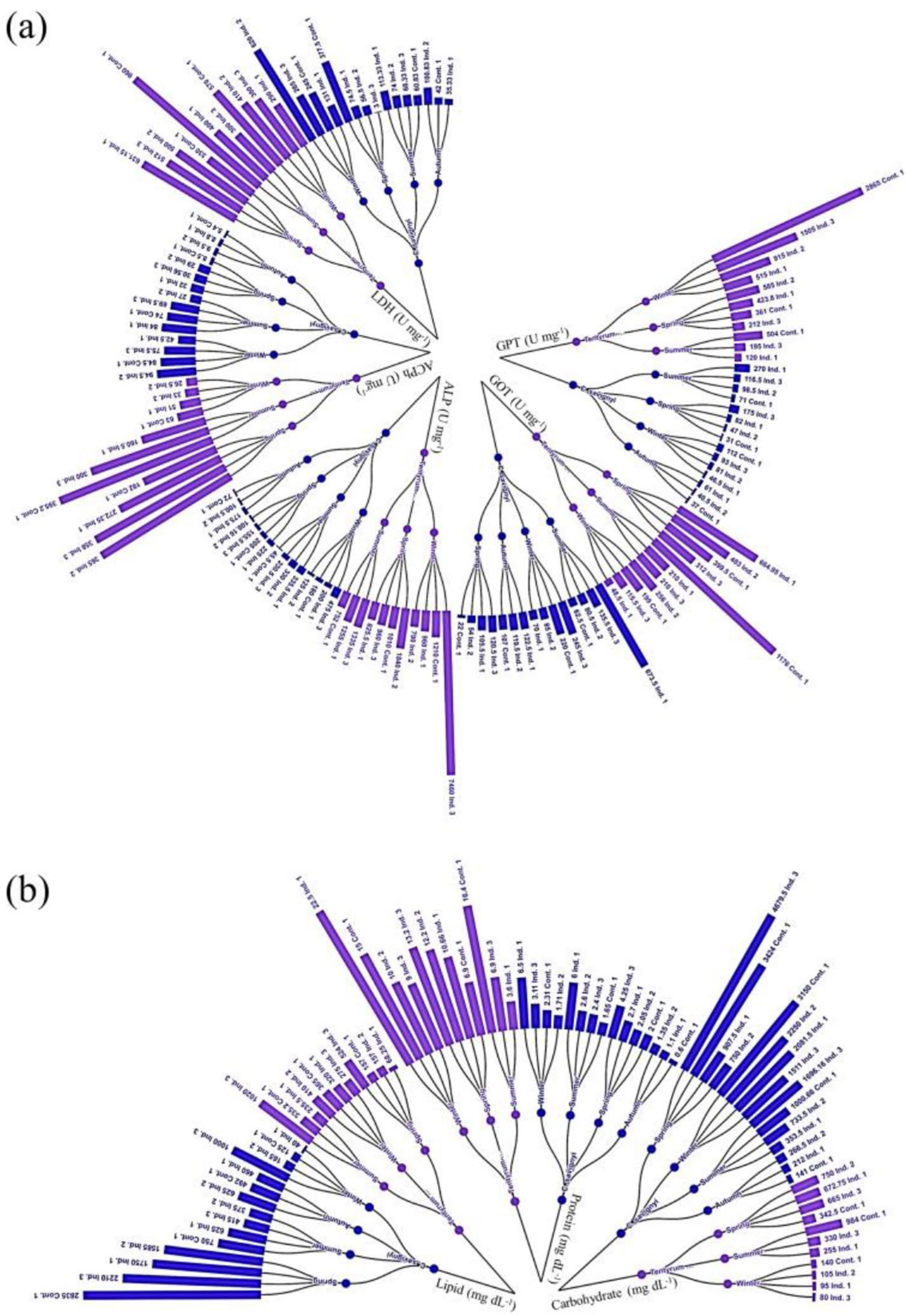


Fig. 5 Radial bar tree represent mean enzymatic values represented for both insect species at different site during studied period

showed statistical significance ($P<0.05$), whereas the latter did not ($P>0.05$). All business locations were not different from the control location in terms of ACPh value ($P>0.05$). When compared to the control site, all industrial locations had P-values for LDH that were more than 0.05. The result for Lipids indicates a statistically significant difference ($P<0.05$) between the experimental and control locations. In comparison to the control site, industrial locations 1 and 3 had significantly higher protein concentrations ($P<0.05$). Carbohydrates also showed statistically significant ($P<0.05$) variation across industrial sites 1 and 2, relative to the control site.

Concern spatial variation of the enzymes, we found that the enzymes GPT, GOT, ALP, ACPh, and protein increased in the polluted industrial sites, while decreasing in the control site, while the LDH, lipid, and carbohydrates decreased or inhibition at industrial sites in comparing to control site. For seasonal variations we found that the activity of enzymes GPT, GOT, ALP, ACPh, lipid, protein, and carbohydrate in hotter seasons were higher than activity in colder seasons. While LDH activity increased in colder seasons.

Regarding the relationship between HM pollution and the role of enzymes in protecting insects from the effect of this pollution, we found that the enzymes (GPT, ALP, ACPh, and protein) are affected by an increase in the concentration of chromium, while the enzymes of ACPh, lipid, and carbohydrates are affected by an increase in Cd, Cu, and Zn.

Enzymatic biomarker in Tentyrum sp Seasonal variation in the activities of the enzyme as GPT, GOT, ALP, ACPh, LDH and main metabolites (lipid, protein, carbohydrate) were detected in *Tentyrum sp* at Borg El-Arab (Fig. 5).

Industrial locations differed considerably ($P<0.05$) compared to the control site considering GPT. Industrial sites 1 and 3 showed statistically significant differences in GOT from the control site, but industrial site 2 showed no such differences ($P>0.05$). Industrial 3 was significant ($P<0.05$) with the control site for ALP, whereas industrial 1, 2 were not significant ($P>0.05$). When compared to the control group, the reported ACPh levels at every given industrial location were not different. Sites in industry 1 and 3 LDH values were statistically significant compared to the control ($P<0.05$), whereas site 2 was not ($P>0.05$). In comparison to the control site, only the third industrial site had a lipid value that was statistically significant ($P<0.05$), whereas the first and second industrial sites were not ($P>0.05$). When compared to the control group, the recorded protein value at each given industrial location was not different from the norm ($P>0.05$). Carbohydrates saw significant differences ($P<0.05$) between the control and industrial 1 and 2 locations. Compared to the control site, industrial 3 did not significantly differ ($P>0.05$).

Concern spatial variation of the enzymes, we found that the enzymes GPT, GOT, ACPh, LDH, protein, and carbohydrate decreased or inhibition in the industrial sites, while increasing in the control site, while the ALP, and lipid increased at industrial sites in comparing to control site. For seasonal variations we found that the activity of enzymes (GOT, ACPh, LDH, and carbohydrate in hotter seasons were higher than activity in colder seasons. While GPT, ALP) and lipid and protein activity increased in colder seasons.

Regarding the relationship between HM pollution and the role of enzymes in protecting insects from the effect of this pollution, we found that the enzymes (GPT, GOT, ALP, and lipid) are affected by an increase in the concentration of Cu, and Cd, while the concentration of carbohydrate is affected by an increase in Cd, Cu, Cr, and Zn.

3.5 Stepwise regression between HM and enzymatic activity in *C. savignyi* and *Tentyrum sp* at Borg El-Arab district

A stepwise regression analysis is used to minimize the independent variables (HMs) which affect or do not affect the dependent variable (Enzyme activity). stepwise regression equations had been built to produce estimated equations that can be used to predict the future conditions of any changes occurred in the dependent variable (Enzyme activity) because of changing in the independent variables (HMs). The relationship revealed that Cd influences GPT, ACPh, protein, and carbohydrate of *C. savignyi*, and Cu influences ALP, while Cr and Zn influence lipid levels of *C. savignyi*. Meanwhile no effect observed for any HM on GOT and LDH in *C. savignyi.* Cd influences ACPh and carbohydrate of *Tentyrum sp*, and Cd, Cr influence GPT of *Tentyrum sp*, while Cr influences lipid levels, and Cd, Cr, Cu influence protein, meanwhile no effect observed for any HM on GOT, ALP, and LDH in *Tentyrum sp* (Table 2).

CCA revealed the role effect of HM evels within investigated species on their enzyme's activity, showing that HM ffect with different power on the enzymatic activity of both species, leading into the present paper conclusion as insects could be utilized as biomarker for contaminated environment (Fig. 6).

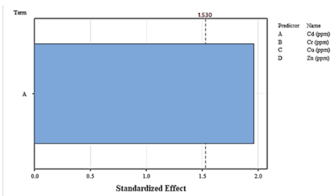
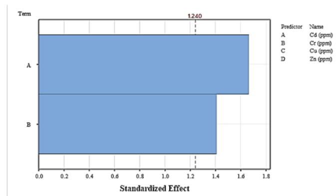
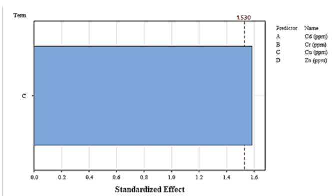
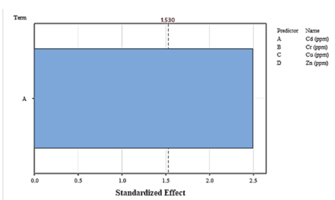
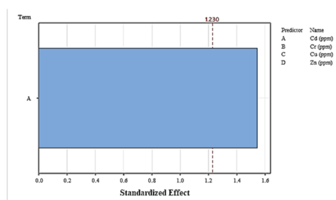
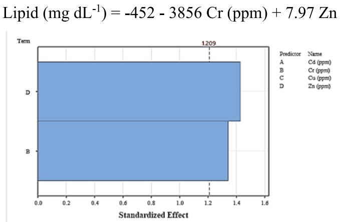
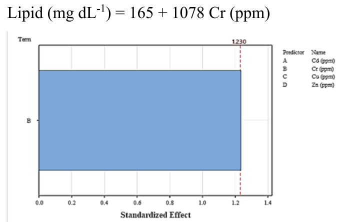
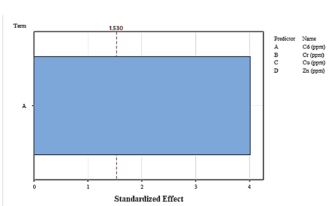
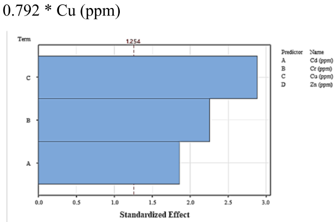
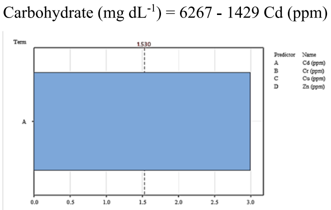
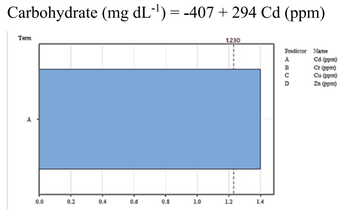
Table 3 summarize the biomarker responses of the two insect species, emphasizing their effectiveness as indicators of pollution, highlighting the present study findings and clearly indicate the direct effect of the contamination to the insect enzymatic profile.

4 Discussion

The soil has been polluted due to the presence of HMs. This may result in the transmission of the contaminants to various levels of the trophic chain [43]. Pollution is one of the most prominent human causes of environmental change. Polluting terrestrial habitats with HMs from factories and cars has ecotoxicological consequences for many insect species [44].

In the study conducted in Borg El-Arab, HM accumulation in soil was investigated, and the results were compared between industrial sites and control sites.

Table 2 Stepwise regression of enzyme activity of *C. savignyi* and *Tentyrum sp* in relation to heavy metal concentration in the observed sites

	C. savignyi	*Tentyrum sp*
GPT (U mg^{-1})	GPT (U mg^{-1}) = 254.4 - 49.5 Cd (ppm)	GPT (U mg^{-1}) = 3649 - 833 Cd (ppm) - 3526 Cr (ppm)
		
GOT (U mg^{-1})	No heavy metals represent any effect on GOT activity	
AlP (U mg^{-1})	AlP (U mg^{-1}) = 401 - 6.11 Cu (ppm)	No heavy metals represent any effect on ALP activity
		
ACPh (U mg^{-1})	ACPh (U mg^{-1}) = 144.7 - 30.2 Cd (ppm)	ACPh (U mg^{-1}) = -199 + 145.3 Cd (ppm)
		
LDH (U mg^{-1})	No heavy metals represent any effect on LDH activity	
	Lipid (mg dL^{-1}) = -452 - 3856 Cr (ppm) + 7.97 Zn (ppm)	Lipid (mg dL^{-1}) = 165 + 1078 Cr (ppm)
Lipid (mg L^{-1})		
Protein (g L^{-1})	Protein (g dL^{-1}) = 9.57 - 2.080 Cd (ppm)	Protein (g dL^{-1}) = 16.28 - 6.80 Cd (ppm) - 34.0 Cr (ppm) + 0.792 * Cu (ppm)
		
	Carbohydrate (mg dL^{-1}) = 6267 - 1429 Cd (ppm)	Carbohydrate (mg dL^{-1}) = -407 + 294 Cd (ppm)
Carbohydrate (mg L^{-1})		

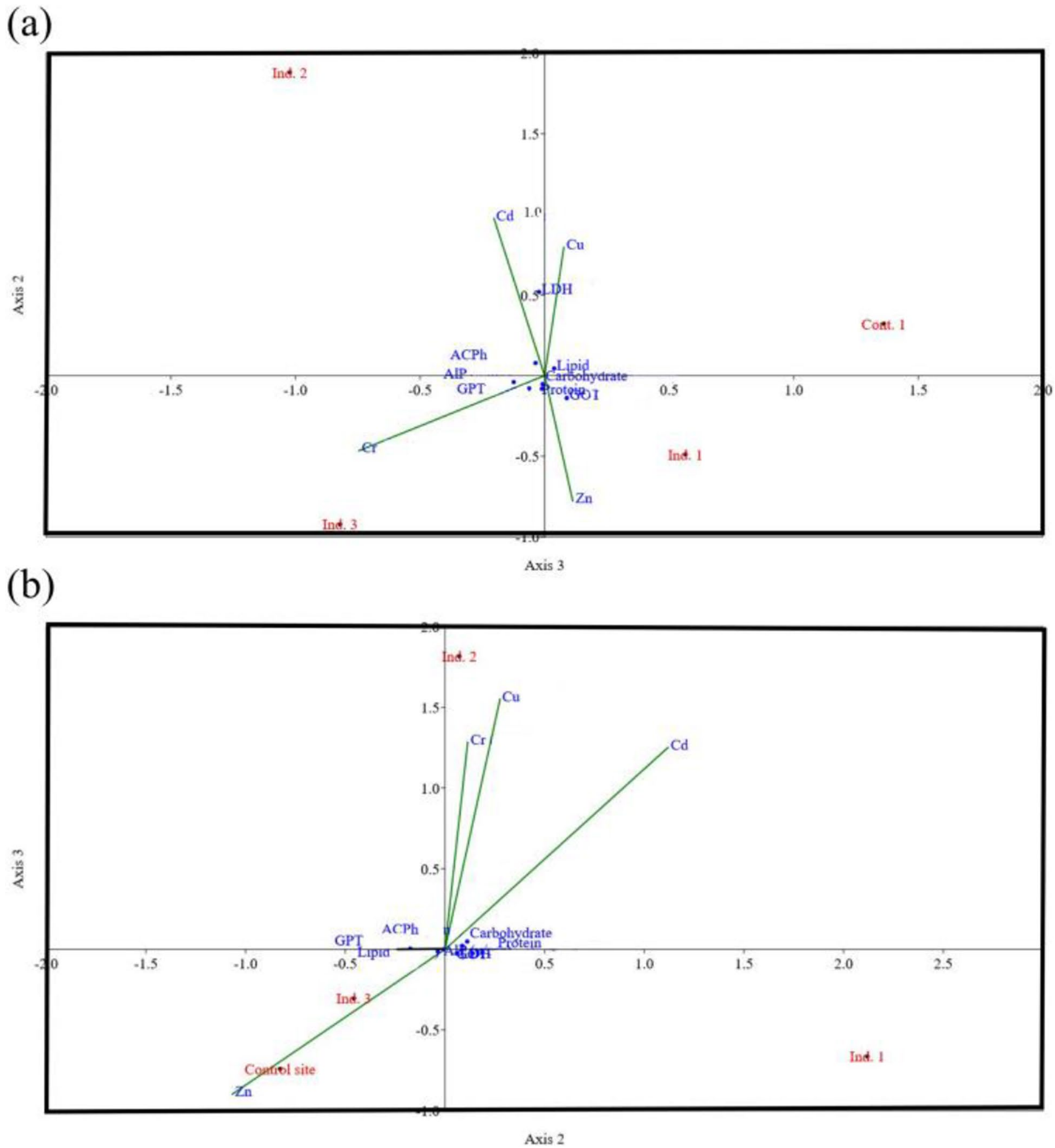


Fig. 6 CCA correlation between (**a**) Soil heavy metals relation to enzymes activity in *C. saviginyi* (**b**) Soil heavy metals relation to enzymes activity in *Tentyrum sp*

According to the findings of the current research, the accumulation of HMs in soil was shown to occur in the following order, beginning with the highest concentrations and ending with the lowest: When compared to Cr, Cd, and Cu, Zn is higher. In addition, it was discovered that the soil and sediment were extensively polluted with a range of metals that were derived from industrial effluent. These metals include Cd and Zn, which eventually have an effect on the physiological parameters of organisms that are located in the ecosystem of the soil. This Zn finding is consistent with the findings of Cui et al. [45]. This agrees with the findings of 11. Soliman

Table 3 A summary table that compares the biomarker responses of the two insect species, emphasizing their effectiveness as indicators of pollution

Biomarker	*Cataglyphis saviginyi*	*Tentyrum Sp*	Effectiveness as Indicators
Glutamic Pyruvic Transaminase (GPT)	Increased activity in industrial sites	Decreased activity in industrial sites	*C. saviginyi* shows higher sensitivity to pollution with elevated GPT levels in contaminated areas
Glutamic Oxaloacetic Transaminase (GOT)	Increased activity in industrial sites	Decreased activity in industrial sites	*C. saviginyi* exhibited stronger responses, indicating its higher sensitivity as a bioindicator
Alkaline Phosphatase (ALP)	Increased activity in industrial sites	Increased activity in industrial sites	Both species showed similar increases in ALP, making them effective in indicating environmental pollution
Acid Phosphatase (ACPh)	Increased activity in industrial sites	Decreased activity in industrial sites	*C. saviginyi* showed higher ACPh activity, making it a more effective biomarker for industrial pollution
Lactate Dehydrogenase (LDH)	Decreased activity in industrial sites	Decreased activity in industrial sites	Both species showed similar suppression of LDH, indicating its reliability as a pollution biomarker
Protein	Increased concentration in industrial sites	Decreased concentration in industrial sites	*C. saviginyi* exhibited a stronger increase, making it a more responsive bioindicator for protein changes
Lipids	Decreased concentration in industrial sites	Increased concentration in industrial sites	*Tentyrum Sp* showed a better response to lipid accumulation, making it more sensitive to lipid-based pollution biomarkers
Carbohydrates	Decreased concentration in industrial sites	Decreased concentration in industrial sites	Both species showed similar trends, indicating effective use as carbohydrate biomarkers

and El-Shazly [11], who found that metal concentrations increased in close proximity to points of industrial pollution. The findings showed that Zinc (1468 ± 16), Chromium (44 ± 5), Cadmium (4 ± 0.14), and copper (1 ± 0.0) were found in the soil at the highest concentrations at the areas of industrial 3. According to the data, there is a statistically significant link between the aforementioned metals and the locations of the studies, corroborated by the findings of Bream et al. [46].

Because of their proximity to the soil's harmful substances, invertebrates are excellent indicators of the impact of human activities on the terrestrial environment [47]. Numerous bug species have the capacity to store environmental toxins in their bodies [48]. Thus, ecotoxicological investigations that use insects as bioindicators are of tremendous interest. Both *C. savignyi* (Hymenoptera: Formicidae) and *Tentyrum Sp*., two types of terrestrial insects, accumulated heavy metals in the present research (Coleoptera: Tenebrionidae). Because of their nesting habits, ants are well-suited to detecting the accumulation of heavy metals; this makes them useful for assessing evolutionary processes, monitoring the environmental impact of metals derived from human activities, and detecting changes in morphological traits, as explained in greater detail by Grze [49]. That's why ants have a bad rep for hoarding toxic metals [12]. In particular, *C. savignyi* was selected for this investigation because of its prevalence across all site types, low population density, moderate colony size, and high activity [18]. This aggregation was also detected using *Tentyrum Sp*. (Coleoptera: Tenebrionidae). The behavior of tenebrionids is strongly tied to anthropogenic-induced landscape transformation, as Bhargava [50] and Fattorini [51] explained. Tenebrionids are characterized by their existence in a broad range of habitats across all biogeographical areas. Tenebrionids have recently been shown to be sensitive monitors for cadmium soil pollution, making them a useful bio-monitor and bioindicator for soil pollution according to research by Grzes [49]. According to the findings of our research, there was a reasonably wide range for each metal that was investigated in terms of the amount of selected HMs that were found in the total bodies of a variety of insects. According to Cain et al. [52], it is preferable to use whole-body detection of HMs since it more accurately represents the greater concentrations of HMs een in insects. Zinc and Cupper also showed a greater concentration than the majority of the elements, notably Chromium. A similar trend holds true for the quantity of these metals in soil. This makes sense, since it agrees with the findings of Jelaska et al. [53], who found a correlation between the elemental content of soil and the amounts of copper and zinc found in studied insects. While Bream et al. [46] attributes the accumulation of HMs in aquatic insects to an increase in total dissolved metals in water or sediment, Azam et al. [54] attribute the accumulation of HMs in terrestrial insects around industrial area of Gujrat to an increase in metals in soil.

Seasonal variation of HMs accumulation at different study species is represented. Spring is characterized as being the highest season in the concentration of total HMs related to mainly increasing the concentration of Zn and Cu at the study area. This result is agreeable with the results of Yahaya et al. [55] and Phuong [56] who verified that HM content in soil is greater when it is dry than when it is wet, and Yahaya et al. [55] explained this outcome by the fact that HMs are washed away during the rainy season. Finally, each species in the research reflects the buildup of HMs in its own unique way, making them effective bioindicator species for tracking pollution from factories. The ants' observation supports this conclusion [12], beetles [57] as a good bioindicator for metal pollution.

The detection of HM accumulation at distinct species is crucial for learning about the concentration of HMs there, but the state of pollution and its degree was clarified using several pollution indicators.

The following is a list of the results that the CF has produced for heavy metals: Zn > Cd > Cr > Cu. The industrial 3 setting had the highest level of Zn contamination, while the other two settings and the control group had even mild levels of contamination. The highest level of contamination was discovered in the industrial 3 setting. It is the industrial 3 site that is the most contaminated in terms of cadmium pollution, while the other two industrial sites and the control site are just marginally more contaminated than the industrial 3 site. To summarize, the levels of contamination for Cr and Cu are relatively modest throughout the board. The computed values of Cdeg in soil samples range from 5.2 to 27.1, with Cdeg at industrial 3 indicating a high degree of pollution compared to Cdeg at industrial 1, 2, and the control, all of which indicate a low degree of contamination.

Igeo was estimated for all metals using the background values given by Taylor and McLennan [31] since there is no background value specified for these elements in this city. Zn topped Cd, Cr, and Cu on the Igeo for the HMs tested.

The risk factor was utilized to assess the level of soil contamination by HMs. According to Hakanson's classification, an Er value below 40 indicates low risk, between 40 and 80 indicates moderate risk, between 80 and 160 indicates considerable risk, and values above 160 indicate high ecological risk. In our study, zinc posed a high ecological risk at all sites, with Er values ranging from 229 to 1469, while industrial site 3 exhibited an extremely high risk. Chromium's Er values ranged from 15 to 45, representing a moderate risk at most sites, though it posed a significant risk at industrial site 3. Both cadmium and copper had Er values below 40, suggesting negligible ecological risks for these metals across the sites.

According to the metric known as the mCd, the level of contamination is highest in the Industrial 3 category, lowest in the Industrial 1 and Control categories, and lowest in the Industrial 2 category.

PLI readings varied from 0.45 to 1.3, with values below 1 indicating no pollution and values between 1 and 2 indicating moderate pollution [58]. According to PI indices industrial 3 was at heavy pollution, meanwhile industrial 1 and control were moderate pollution, but industrial 2 exhibits a slight pollution. The dissimilarities emerge because the indices respond to soil contaminants in different ways [59].

To confirm the impact of soil HM pollution on the bodies of *C. savignyi* and *Tentyrum Sp*., we examined the BAF between the soil and the metal accumulations (in ppm) in the insect bodies from the investigated sites. The present work categorizes *C. savignyi*. BAF was shown to be a macro-concentrator for Cd at all sites, except in industrial 3 where it acted as a de-concentrator and released the metals into the soil. Regarding Cr and Zn at all investigated sites, BAF served as a de-concentrator. All sites exhibited macro-concentration of BAF for Cu. For Cd in *Tentyrum Sp*, the results showed that BAF acted as a micro-concentrator at industrial 1 and control site, whereas at industrial 2 it acted as a macro-concentrator and de-concentrator against industrial 3. At all sites, BAF served as a de-concentrator for Cr and Zn. For copper, BAF acted as a macro-concentrator at all sites. Clearly as stated by Okrutniak and Grzes [14], in heavily contaminated locations, the BAF diminishes with increasing soil metal content. This refers to a non-linear connection between metal concentration in the environment and ant bodies, implying that ants may collect metals to a specific level beyond which the regression line hits a plateau.

Enzyme activity which is particular to tissues has been used to identify organ and tissue dysfunction caused by chemical toxicity. Phosphatases and transaminases are very diagnostically valuable because of their involvement in multiple physiological processes within the insect body. The objective of this study was to investigate the impact of industrial pollution on *C. savignyi* and *Tentyrum Sp* by evaluating the activity of various enzymes. Experimental studies have demonstrated that *C. savignyi* at industrial sites significantly increases the activity of GPT, GOT, ALP, ACPh, and protein in comparison to control groups. This outcome is consistent with Shonouda et al. [60] who found elevated levels of GPT and GOT activity in *Anaceana globulus* tissues from heavily polluted locations. In addition, lufenuron and chlorfluazuron therapy resulted in increased GPT and GOT levels in *S. littoralis* tissues [61]. Increased GPT and GOT activity following exposure to pollution may be the consequence of a combination of proteolytic protein breakdown and the downregulation of protein synthesis [62]. Meanwhile, the activity of LDH, lipids, and carbohydrates at industrial sites is comparable to control. And this resulted in conflict with Bream et al. [18] who found that LDH increased at industrial sites. On the other hand, *Tentyrum sp* increases the activity of ALP and lipid at industrial sites compared with control, while decreasing the activity of GPT, GOT, ACPh, LDH, protein, and carbohydrate at industrial sites compared with control.

Considering seasonal fluctuations Studies on *C. saviginyi* revealed that the enzymatic activity of GPT, GOT, ALP, ACPh, lipid, protein, and carbohydrate was greater during warmer seasons compared to colder seasons. LDH activity exhibited an increase during colder seasons. Conversely, in *Tentyrum Sp*, the enzymatic activity of GOT, ACPh, LDH, lipid, and carbohydrate was greater during warmer seasons compared to colder seasons. The activity of GPT, ALP, and protein exhibited a rise during colder seasons.

The relationship between HM contamination and the function of enzymes in safeguarding insects against the impact of such contamination is examined. Our study in *C. saviginyi* revealed that the enzymes GPT, ALP, ACPh, and protein are influenced by higher chromium concentrations, whereas the enzymes ACPh, lipid, and carbohydrates are influenced by higher levels of Cd, Cu, and Zn. Investigating *Tentyrum Sp*, we observed that the enzymes GPT, GOT, ALP, and lipid are influenced by higher levels of Cu and Cd, while the concentration of carbohydrates is influenced by higher levels of Cd, Cu, Cr, and Zn.

The enzymatic biomarkers of *C. saviginyi* and *Tentyrum Sp* exhibit metabolic alterations that provide insight into the physiological stress experienced by these organisms as a result of HM pollution in the soil. The heightened activity of detoxifying enzymes, namely GPT and GOT, indicates that these insects are undergoing metabolic adaptations to accept the higher concentrations of Zn, Cd, Cr, and Cu present at the industrial locations. These enzymes are crucial in the process of detoxifying toxic chemicals and serve as a reliable indicator of oxidative burden in organisms that have been exposed to them. Specifically, the inhibition of LDH function in both insect species indicates possible disturbances in energy metabolism, as LDH is essential for sustaining anaerobic energy generation during stressful situations.

C. saviginyi and *Tentyrum Sp* exhibit biochemical alterations that indicate physiological stress caused by HM pollution. These changes are characterized by heightened detoxifying enzyme activity (GPT and GOT) and reduced LDH activity, suggesting oxidative stress and disturbances in energy metabolism. The observed alterations indicate metabolic adaptations in reaction to elevated concentrations of Zn, Cd, Cr, and Cu. From an ecological perspective, these disturbances can result in a decrease in insect populations, which in turn might impact the process of nutrient cycling, soil aeration, and the food web. Consequently, both species diversity and the overall health of the ecosystem may be diminished. Primary predators that consume polluted insects may suffer secondary poisoning, resulting in impaired health and reproductive performance. The bioaccumulation of HMs in insects and their subsequent passage up the food chain via pest-contaminated crops provide significant hazards to human health, including renal impairment and cancer. Hence, it is imperative to promptly identify and track pollution by utilizing insect bioindicators to safeguard ecosystems and human communities.

Lastly, the present study has pragmatic implications for legislators, environmental agencies, and community organizations. The findings can be used by policymakers to justify more stringent industrial rules and establish early warning systems utilizing insect biomarkers. Environmental authorities can harness the potential of *C. savignyi* and *Tentyrum Sp* for economical and immediate monitoring of soil contamination, therefore facilitating prompt actions to ecologically vulnerable regions. Using these findings, community groups can promote awareness of the environmental effects of industrial pollution and advocate for sustainable practices to save local ecosystems and public health.

5 Conclusions

The present study demonstrated that HMs in soil detected their accumulation order from high to low concentrations as follows: Zn > Cr > Cd > Cu and all HMs varied significantly for industrial 1 and 3 compared to the control site. Regarding the spatial variation bioaccumulation for *C. saviginyi* and *Tentyrum sp*, all the HMs in the different industrial sites were higher than in the control or natural sites. On the other hand, for seasonal variation all HMs in colder seasons were higher than in hotter seasons, except Zn was a high concentration in hotter seasons regarding *C. saviginyi*. In contrast, for *Tentyrum sp* all HMs in hotter seasons were higher than in colder seasons, except Cu was a high concentration in colder seasons. Calculated Cdeg, mCd, PI and PLI for industrial site 3 revealed a very high degree of contamination, which can be attributed to the increase in industrial activity resulting from the chemical and silicate factories that characterize this region. The BAF increases with a decrease in HMs in soil. The current results highlight the inhibition of LDH, lipid, and carbohydrate levels in *C. saviginyi* at the industrial site compared to the control site. In contrast, GPT, GOT, ALP, ACPh, and protein activity increased. On the other hand, *Tentyrum sp* decreases the activity of GPT, GOT, ACPh, LDH, protein, and carbohydrate at industrial sites compared with control. While increasing the activity of ALP and lipid at industrial sites compared with control Thus, antioxidant enzymatic activities and main metabolites serve as useful biomarkers for assessing and monitoring environmental contamination using insects as bioindicators. Future studies should prioritize the long-term surveillance of HM buildup and enzymatic reactions in bioindicators to comprehend the temporal trajectory of pollution patterns. Broadening the scope of bioindicators to encompass additional insect species from diverse ecological functions would provide a more thorough evaluation of ecological well-being. Furthermore, it is essential to evaluate the wider consequences of pollution on biodiversity, such as alterations in species diversity and community composition. Furthermore, research should investigate the impact of specific contaminants on the biochemical and physiological reactions of insects to gain a deeper understanding of detoxification processes and insect tolerance.

6 Limitation

The present analysis acknowledges several constraints that could potentially impact the data interpretation. Potential confounding variables, such as environmental parameters (e.g., wind, precipitation, and soil characteristics), could have affected the levels of pollutants in both soil and insect samples. The efficacy of Cataglyphis saviginyi and *Tentyrum Sp* as bioindicators is constrained by their limited representation of the environment, and their responses from other species may exhibit variability. Seasonal fluctuations also contributed to the occurrence of higher levels of heavy metal concentrations and metabolic activities during warmer months, which posed challenges in distinguishing these effects from the impacts of pollution. Furthermore, several enzymatic assays can be affected by biological variables that are not directly connected to heavy metal exposure, therefore introducing differences in the obtained results. To enhance the validity of the results, future studies should take into account a wider range of bioindicators, implement longer-term monitoring, and establish more controlled experimental circumstances.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Conceptualization, Ahmed S. Bream; Data curation, Mohamed A.M. El-Tabakh; Formal analysis, Mohamed A.M. El-Tabakh; Funding acquisition, Yasser I. Hamza; Investigation, Yasser I. Hamza; Mohamed A.M. El-Tabakh & Mohammed A. Mahmoud; Methodology, Yasser I. Hamza; Resources, Yasser I. Hamza; Review & editing, Ahmed S. Bream & Mohamed A. M. El-Tabakh. All authors have read and agreed to the manuscript publication.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The authors confirm that the conducted research was by the ethical guidelines and international regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

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