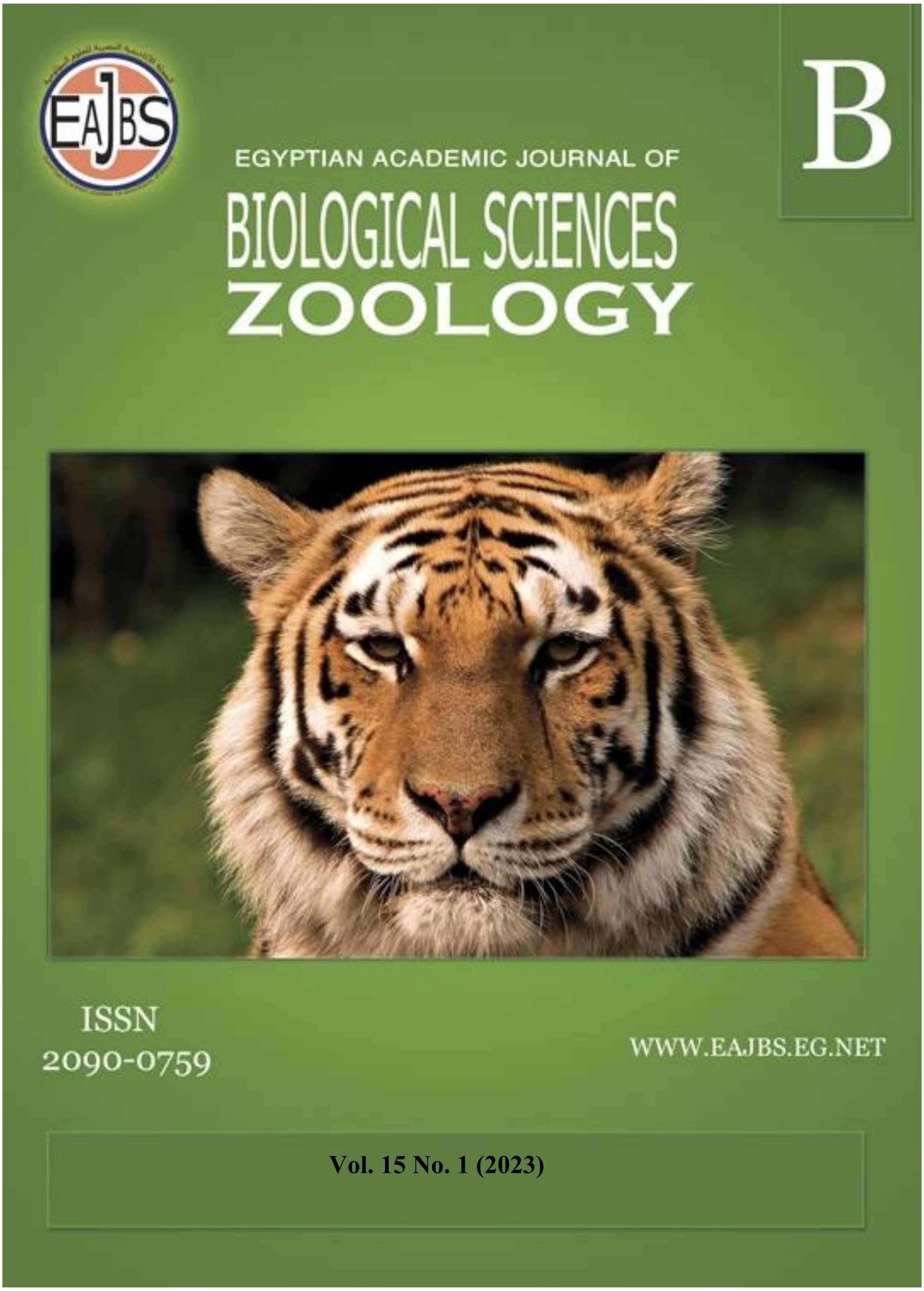

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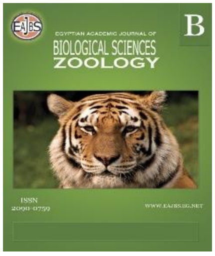

Pollution Indices, Floral Coverage and Newly Enzymatic Biomarker for Soil Heavy Metal Contamination Due to Industrial Activities At 6th October City, Egypt

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ABSTRACT

The current study aims to investigate the environmental effect of industrial activities on floral coverage within the major industrial district of 6th October City, Egypt. Additionally, assess the Detoxification enzymatic activity of *Cataglyphis saviginyi* as a pollution biomarker. To achieve this objective, soil samples on seasonal bases were collected from the investigated areas to assess heavy metal concentrations. Furthermore, seasonal specimens of *C. saviginyi* were collected to study the enzymatic activity of acetylcholinesterase (AChE) and glutathione-S-transferase (GST). Heavy metal contamination pollution indices were calculated, and Nine plant species were identified at investigated study sites for four successive seasons from 2020 to 2021. The accumulation of heavy metal contamination in soil at investigated sites followed the order: As >Ni > Pb > Hg. Heavy metal contamination was significantly higher at industrial locations than at the control site. Pollution indices were calculated in study sites, with Cf and Igeo indices highlighting the contamination of minerals, ordered as Hg > As > Ni > Pb from high to low. Calculated Cdeg and PLI for industrial 1 and 4 revealed a very high level of pollution, attributed to the increase in industrial activity resulting from the chemical, Ceramic, Paints, and metallic industries that characterize this region. The current results highlight the inhibition of GST levels in *C. saviginyi* at the industrial site compared to the control site. In contrast, AChE activity increased. Consequently, the antioxidant enzymatic activities are useful as biomarkers for assessing and monitoring environmental contamination. In conclusion, to our knowledge, this is the first enzymatic activity of *C. saviginyi* evaluated as a bioindicator for industrial pollution in study sites.

INTRODUCTION

Human activities such as urban development, industrial expansion, transportation, excessive application of fertilizers, insecticides, and pesticides, and inadequate management of sewage and waste materials that contain harmful chemicals, along with natural processes such as erosion, weathering, and precipitation, contribute to the accumulation of these substances in soil and water (Bhavana *et al*., 2009). On the other hand, Metals such as Fe, Pb, Cd, and Cr are critical micronutrients for plants and microbes. Heavy metal contamination is a significant environmental issue because of its harmful

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effects and build-up throughout the food chain. The electroplating, painting, and surface treatment industries mostly cause heavy metal contamination. (Shrivastava and Mishra, 2011).

All heavy metals in soil are inaccessible to soil organisms. Heavy metal bioavailability is determined by their concentration in soil solution. (Gambrell, 1994). Their concentration in soil is associated with biological and geochemical cycles (Santos *et al*., 2005; Ebong *et al*., 2007; Saeedi and Amini, 2007). To reduce contaminated site hazards, avert future soil degradation, and define remedial plan techniques, the quantity and toxicity of soil pollution must be measured (Liu *et al*., 2020; Mazurek *et al*., 2019). Pollution indices are useful tools for determining the quality of the soil and identifying sources of pollutants. They may also help with management and remediation activities (Mazurek *et al*., 2019).

On the other hand, Plants can absorb excessive amounts of heavy metals from the soil, which may be hazardous. (Monni *et al*., 2001) and transmitted through the food chain (Wuana and Okieimen 2011; Opaluwa *et al*., 2012). Plants are regarded as an "early warning" sign of stress symptoms caused by pollution. Since heavy metals tend to accumulate in their tissues and fluids, especially (Białońska and Dayan, 2005), As a result, it looks to be incredibly promising for removing toxins from the environment and might be utilized as a bioindicator to detect pollution levels (Garbisu and Alkorta, 2003; United Nations Environment Programme (UNEP), 2010).

Anthropogenic activities are causing changes in natural plant communities in Egypt from ancient times by draining lakes and marshes and reducing species number in aquatic communities (Kassem *et al*., 2019). Grazing, road construction, overexploitation, solid waste, soil salinity, industry, urbanization, and military are the basic human activities that cause vegetation and transformation changes and destruction of habitat in dry and semi-arid areas. regarded as an activity (Moustafa *et al*., 1999; Hussein *et al*., 2021). Changes in climate and land cover, such as cover, height, biomass, humidity, soil temperature, moisture, productivity, and eroding, all influence the structural characteristics of plants in terrestrial ecosystems (Ostberg *et al*., 2015). Human activities such as land cover change, invasive species introduction, overharvesting, and contamination all have an impact on biodiversity reduction (Titeux *et al*., 2016; Pereira *et al*., 2012; Zhu *et al*., 2019). Climate change impacts plant growth, and human activities can modify the interactions between the atmosphere and biosphere, influencing the hydrological cycle either directly or indirectly (Wu *et al*., 2017; Kong *et al*., 2018). Effects of climate change, such as declining soil fertility, intense winds, higher evaporation rates, elevated temperatures, and heavy rains, cause drastic changes in the composition of plant species communities. (Hussein *et al*., 2021; Abd El-Wahab, 2016).

Biochemical indicators have been widely used to assess organism susceptibility to chemical contamination and reviewed by (Min and Kang, 2008; Wilczek *et al*., 2008; Praet *et al*., 2014). They also have a wide range of enzyme activities that allow them to metabolize or degrade various xenobiotics (Wilczek *et al*., 2004; Augustyniak *et al*., 2005; Wilczek *et al*., 2008). Furthermore, Enzymes shield cells from oxidative stress, which suppresses the activity of antioxidants. As a result, more crucial molecules like DNA, RNA, and proteins are protected (Augustyniak and Migula, 2000). GST enzyme is also thought to be an antioxidant, facilitating their conjugation with GSH and producing non-toxic compounds (Xu *et al*., 2015). Acetylcholinesterase (AChE) is an enzyme that helps in the transmission of nerve signals by breaking down acetylcholine into choline and acetate through a hydrolysis reaction (Kim and Lee, 2018). At first, it was employed as a biomarker for a variety of dangerous compounds such as carbamate and organophosphate. However, other xenobiotics, such as heavy metals, can have an effect on its action (Praet *et al*., 2014; Bonnail *et al*., 2016).

The basic aims of this research were to measure soil contamination, pollution degree, and contamination risk with heavy metal ions using various pollution indices on the 6th of October City, Egypt. and investigate ecological indicators to assess the industrial emissions impact on the vegetation coverage at the surrounding industrial area. Finally, the activity of detoxifying enzymes in *Cataglyphis saviginyi* was detected as bio-indicators for contamination.

MATERIALS AND METHODS

Study Area:

6th October city is one of the new cities, located in Cairo desert edge, approximately 32 kilometers from Cairo governorate, near the junction of longitude (30º, 45′) in the east and latitude (30º, 00′) to the north. It is also inside the administrative limits of Giza and has a unique geographical location close to the broader Cairo region **(Ramadan 2016).** The localities of the current study will divide industry in comparison to natural or control sites into (Fig. 1):

Control Site: Lies at N 29° 56' 43.2"- E 30° 54' 15.0" at the position of buses neighborhood Sixth of October City, which characterize by no human impact and natural habitat in a desert region.

Industrial Site (1): Lies at N 29° 54' 11.9"- E 30° 52' 56.8" at 6th industrial zone. This site represents the community of several types of factories, such as Elmarakby for metallic industries, October for metal cans, National Plastic Company, and Kimi Art Company.

Industrial Site (2): Lies at N 29° 53' 59.6"- E 30° 53' 05.0" at the 6th industrial zone. This site represents the community of distinct types of factories, such as Egypt for panel and steel industries, Medical Supplies company, Pipe steel, Misr for cold room industries and metal industries and Amec steel laser cutting.

Industrial Site (3): Lies at N 29° 53' 49.4"- E 30° 53' 33.4" at the 5th industrial zone. This site represents the community of several types of factories, such as Pasabahce Egypt S.A.E, Eastern Company for Tobacco, and Uni-Lime factory.

Industrial Site (4): This study site lies at N 29° 54' 21.3"- E 30° 54' 45.4" at the 3rd industrial zone. This site represents the community of diverse types of factories, such as Alfa Ceramicand United Co. For Paints and Chemicals S.A.E - Dry Mix and Venus Company Factory.

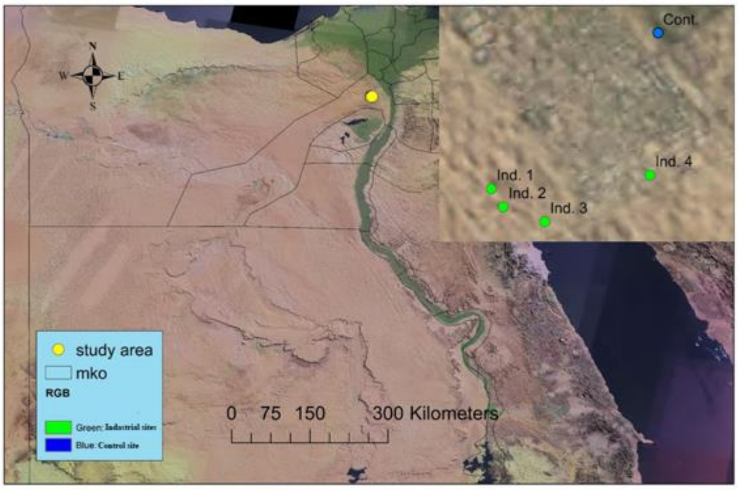


Fig. (1): Map of investigated sites

Climatic Factors:

Climatic data were obtained from the meteorological station on 6th October city. The seasonal mean of average air temperature degree (C^o), Pressure (kPa), wind speed (m s^{-1}), and Rh (%) were the main climatic parameters.

Floral Coverage:

Identifying the plant species associated with the several types of study sites. Therefore, plant species were collected, preserved, and pressed until identification. The nomenclature of the plant species was identified according to key authors (Tackholm, 1947; Boulos, 1999 & 2000 & 2002 & 2005 & 2008) .According to Shukla and Chandel (1989), the relative value of vegetation cover for each species was calculated by counting the cover of each species to the total vegetation cover within a series of randomly distributed stands.

Heavy Metal Analysis:

For estimation of heavy metals in soil, samples of the soil were digested based on the protocol of Perchloric Acid–Nitric Acid Digestion described by **(Hesse 1971)**and analyzed by Varian vista AX CCD Simultaneous ICP-AES and expressed as ppm dry weight.

Pollution Indices:

Different pollution indices were used for the detection of the status of pollution.

Contamination Factor:

The assessment of soil contamination was also conducted using the contamination factor and degree. In the version suggested by Hakanson (1980), they enable an assessment of soil contamination by referencing the concentrations in the surface layer of soil to preindustrial levels.

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

Where Msample and Mbackground are heavy metal concentrations of the studied soil sample and the background, respectively, the Mbackground was taken for As, Cd, Cr, Cu, Pb, Zn, Ni, and Hg were 13.4, 1, 69, 39, 17, 67, 55, and 0.08 mg/kg, respectively (Taylor and McLennan,1995). Hakanson (1980) categorized the contamination values as; $CF < 1$ (low contamination), $1 < CF < 3$ (moderate contamination), $3 < CF < 6$ (considerable contamination) and $CF > 6$ (very high contamination).

Degree of Contamination (CD):

To simplify the control of pollution, Hakanson (1980) presented an investigative tool called the 'degree of contamination' (CD) and proposed a classification for contamination degree as $CD < 6$ (low degree of contamination), $6 < CD < 12$ (moderate degree of contamination), $12 < CD < 24$ (considerable degree of contamination) and $CD > 24$ (high degree of contamination), indicating alarming anthropogenic pollution. It was designated as the summation of the CF of each element concerned as under:

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

Where CF_i is the contamination factor for each element (i) investigated. This index classifies the soil into four categories of contamination that are: Low degree of contamination ($\text{Cdeg} < 8$), Moderate degree of contamination ($8 \leq \text{Cdeg} \leq 16$), Considerable degree of contamination ($16 \leq \text{Cdeg} \leq 32$), Very high degree of contamination ($32 \leq \text{Cdeg}$) Hakanson (1980); Caeiro *et al*., (2005).

Modified Degree of Contamination (mCd):

According to Abrahim and Parker (2008), Machender *et al.*, (2011), and Rahman *et al*., (2012), the modified degree of contamination (mCd) is the average value of pollution indices for all trace elements (Cf_i), provided that at least three chemical elements

(n) are used in the calculations.

Klik *et al.*, (2020) classify the soil on a seven-order scale of contamination, which is: very low contamination (mCdeg ≤ 1.5), Low contamination (1.5 ≤ mCdeg ≤ 2), Moderate contamination (2 ≤ mCdeg ≤ 4), High contamination (4 ≤ mCdeg ≤ 8), Very high contamination (8 ≤ mCdeg ≤ 16), Extremely high contamination (16 ≤ mCdeg ≤ 32), Ultra-high contamination (32 ≤ mCdeg). This index is calculated using the following equation:

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

where *n* is the number of elements evaluated, and CF is the contamination factor for each element (*i*) individually.

Geo-accumulation Index (Igeo):

Igeo has been widely used as a geochemical criterion to assess the contamination level of a particular element in environmental sediments or soils since 1969. The following equation is used to determine Igeo values Muller, (1969):

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

Where C_n is the measured element's concentration in the soil, and Bn is the provided metal's geochemical background value. The constant 1.5 is a background matrix adjustment factor that considers natural oscillations caused by lithogenic influences. In this study, the background values taken for As, Cd, Cr, Cu, Pb, Zn, Ni, and Hg were 13.4, 1, 69, 39, 17, 67, 55, and 0.08 mg/kg, respectively (Taylor and McLennan,1995).

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 (Chen *et al.*, 2015, Hussain *et al.*, 2015, Wei and Yang, 2010).

The Pollution Load Index (PLI):

A pollution load index is a tool used to assess the quality of soil (Liu *et al.*,2005; Santos *et al.*, 2020; Karki and Verma, 2020; Tomlinson *et al.*, 1980), is calculated by the nth root of the multiplication of the contamination factors of the investigated chemical elements, as the following expression:

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

CF_1, CF_2, and CF_n are the contamination factors of 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).

Pollution Index:

The pollution index (PI) is defined as the ratio of the heavy metal concentration to the geometric mean of background concentrations, which is used to calculate the pollution level of individual elements Keshav Krishna and Rama Mohan, (2016); Chen *et al.*, (2005); Sun *et al.*, (2010). The calculation of PI is as follows:

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

Where CFaverage is the average value of the contamination factor, and Cmax is the maximum value of the contamination factors**.** The PI value of each metal of each sample site is calculated and classified respectively as low contamination (PI ≤ 1.0), moderate contamination (1.0 < PI≤ 3.0), or high contamination (PI > 3.0) (Keshav Krishna and Rama Mohan, 2016; Wei and Yang,2010; Chen *et al.*,2005).

Biochemical Analysis of Enzymes:

According to the importance of enzymatic biomarkers in detecting organisms' exposure to chemicals and environmental pollution, which are typically more sensitive and represent early detectable responses to environmental changes (Domingues *et al*., 2010; Praet *et al*., 2014). The current study advocated using specified enzymes such as Acetylcholinesterase enzyme (AchE) and Glutathione-s-Transferase (GST) for pollution monitoring as effective biomarkers in sample preparation and analysis.

Sample Preparation and Analysis:

Whole body insects were weighed and homogenized in a saline solution (1 gm of tissue insect on 1 ml saline solution 0.7 %) using a fine polytron 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.

Acetylcholinesterase:

According to the technique adopted by Knedel *et al*. (1967), samples were assayed using a commercial kit to measure the activity of AchE concentrations by Biodiagnostic Company, Egypt.

Glutathione-S-transferases:

Glutathione-S-transferases activity (GST) was measured according to Habig *et al*, (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.

Statistical Analysis:

The statistical software SPSS V.22 was used to code and input the data. Data were assessed for satisfying assumptions of parametric tests; continuous variables were subjected to Shapiro-Wilk and Kolmogorov-Smirnov tests for normality. Probability and percentile data were standardized for normality using Arcsine Square Root. Data were presented as mean and stander deviation. ANOVA analyses were done for the investigated sites regarding the recorded variables; analysis was evaluated using three replicates at least for each group; post-hoc analysis was assessed using Tukey pairwise comparison using Minitab V 14; P-values were considered significant at<0.05. Regression was conducted to figure out the relation and the prediction equation between heavy soil metals and *C. savignyi* Detoxification enzyme; the analysis became available using Sigma Plot V 14.0. CCA was illustrated using Past V 4.12. Data were visualized when possible, using R studio V 2022.02.4.

RESULTS

Climatic Factors:

Table (1) shows the recorded meteorological data obtained during the current investigation; there was a significant seasonal fluctuation in climatic factors ($P< 0.05$). The highest temperature was 28.63 ± 1.2C^o, during the summer of 2020. On the contrary, winter 2021 was characterized by its lower temperature (14.85 ± 0.54C^o). Conversely, the greatest relative humidity value was recorded during Winter 2021(74.56 ± 2.35%), and the lowest relative humidity (40.14 ± 10.54%) was recorded during Spring 2021. Furthermore, the highest value of wind Speed was recorded during Spring 2021 (2.82±0.41m^{s-1}), and the lowest value (1.42±0.29m^{s-1}) in Autumn 2020. Meanwhile, the maximum value of Pressure was recorded during the autumn 2020 (100.56±0.04kpa), and the lowest value (99.62±0.04kpa) in the Summer of 2020.

Table (1): The microclimatic factors throughout the current study in October, Egypt.

Season	Temp (C°)	R.H (%)	W. Speed (m $^{s-1}$)	Pressure (kPa)
Summer	28.63 ± 1.2^a	48.68 ± 6.08^b	2.74 ± 0.19^a	99.62 ± 0.04^a
Autumn	15.42 ± 0.55^b	69.04 ± 0.73^a	1.42 ± 0.29^b	100.56 ± 0.04^a
Winter	14.85 ± 0.54^b	74.56 ± 2.35^a	$2.05\pm0.46^{a,b}$	100.54 ± 0.2^a
Spring	26.49 ± 1.96^a	40.14 ± 10.54^b	2.82 ± 0.41^a	99.96 ± 0.32^a

**Means in a column that shares the same letter is not significantly different (P> 0.05)*

Floral Coverage:

The diversity and fluctuation of species abundance characterize vegetation coverage. Nine plant species were identified (Table 2) at investigated study sites. *Zilla spinosa* (Family: Brassicaceae), *Tamarix nilotica* (Family: Tamaricaceae) and *Conyza dioscoridis* (Family: Compositae) are the most recorded species in study sites. In special, *Tamarix nilotica* was the dominant species in all vegetation cover at different study sites. Its relative vegetation cover ranged between 20-35%. On the other hand, the October Plant community was characterized by a variety of plant species (9 distinct species), with each of their relative vegetation covering ranging between 5-35% (Fig. 2).

Table (2). Spatial variations of identified plant species (presence; ✓) and their relative vegetation cover (value %) at different study sites in October, Egypt.

Species	Family	Cont.	Ind. 1	Ind. 2	Ind. 3	Ind. 4
Zilla spinosa	Brassicaceae	✓ (30%)	✓ (30%)	✓ (20%)	✓ (30%)	✓ (30%)
Tamarix nilotica	Tamaricaceae	✓ (20%)	✓ (35%)	✓ (35%)	✓ (30%)	✓ (20%)
Conyza dioscoridis	Compositae	✓ (15%)	✓ (10%)	✓ (20%)	✓ (20%)	✓ (10%)
Phoenix dactilifera	Arecaceae	✓ (10%)				✓ (20%)
Arundo donax	Poaceae	✓ (5%)	✓ (15%)	✓ (10%)		
Deverra tortuosa	Apiaceae	✓ (10%)				
Noea murconata	Chenopodiaceae	✓ (5%)				
Bassia muricata	Amaranthaceae		✓ (10%)	✓ (10%)	✓ (15%)	
Francoeuria crispa	Compositae	✓ (5%)		✓ (5%)	✓ (5%)	✓ (20%)

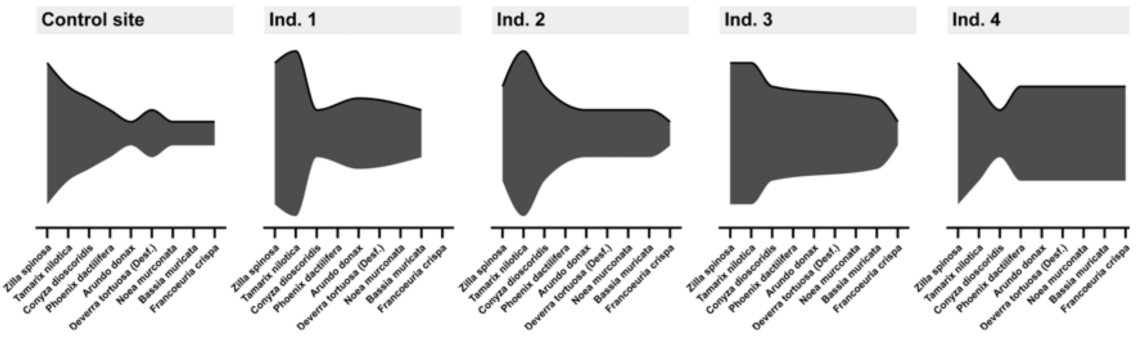
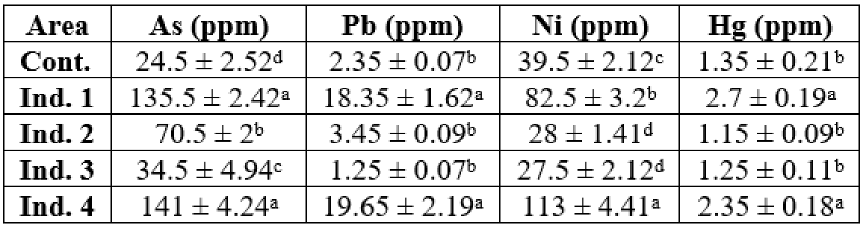


Fig. 2: Stream graph represents recorded floral coverage for study sites.

Heavy Metal in Soil:

Heavy metals in soil were measured **(**Table3**)** as Arsenic (As), Lead (Pb), Nickel (Ni), and Mercury (Hg) at investigated sites. All industrial sites in October significantly differed (P< 0.05) from the control site regarding As, and Ni. Pb and Hg concentrations varied significantly (P< 0.05) between industrial 1 and 4 compared to the control site.

Table 3: Spatial differences in heavy metal (ppm) levels of soil were collected from several industrial sites throughout the current investigation.



Area	As (ppm)	Pb (ppm)	Ni (ppm)	Hg (ppm)
Cont.	24.5 ± 2.52^{d}	2.35 ± 0.07^{b}	39.5 ± 2.12^{c}	1.35 ± 0.21^{b}
Ind. 1	135.5 ± 2.42^{a}	18.35 ± 1.62^{a}	82.5 ± 3.2^{b}	2.7 ± 0.19^{a}
Ind. 2	70.5 ± 2^{b}	3.45 ± 0.09^{b}	28 ± 1.41^{d}	1.15 ± 0.09^{b}
Ind. 3	34.5 ± 4.94^{c}	1.25 ± 0.07^{b}	27.5 ± 2.12^{d}	1.25 ± 0.11^{b}
Ind. 4	141 ± 4.24^{a}	19.65 ± 2.19^{a}	113 ± 4.41^{a}	2.35 ± 0.18^{a}

**Means in a column that shares the same letter is not significantly different (P> 0.05).*

Pollution Indices:

Contamination Factor (CF):

For As, industrial 1 and 4 represent a very high contamination factor, while Industrial 2 shows a considerable contamination factor, Industrial 3 and the control site revealed a moderate contamination factor. For Pb and Ni, all sites show low contamination except industrial 1 and 4exhibit a moderate contamination factor. Finally, Hg at all sites exhibits a high contamination factor (Fig. 3, *a*).

Ecological Risk Factor (E$_r$):

The risk factor was successfully used for assessing the contamination of soils in the environment by heavy metals. Regarding As, industrial 1 and 4 were at considerable ecological risk; Industrial 2 exhibited a moderate ecological risk, while Industrial 3 and the control site were at low ecological risk. For Pb and Hg, all sites represent a low ecological risk. Regarding Ni, all sites present a low ecological risk except for Industrial 1 and 4, which were at considerable ecological risk (Fig. 3, *a*).

Geo-Accumulation Index (Igeo):

Regard As, industrial 1, 2 and 4 were moderately polluted, while Industrial 3 and the control site were unpolluted to moderately polluted. For Pb, all sites were unpolluted, except Industrial 1 and 4 were unpolluted to moderately polluted. Regarding Ni, all sites were unpolluted to moderately polluted. Finally, Hg at all sites was in a moderately polluted state (Fig. 3, *a*).

Degree of Contamination (Cdeg):

Degrees of contamination at industrial 1 and 4 were at a very high degree of contamination, while industrial 2, 3, and the control site were at a considerable degree of contamination (Fig. 3, *b*).

Modified Degree of Contamination (mCd):

Industrials 1 and 4 show a very high degree of contamination; in the same manner, industrial 2, 3, and control sites show a high degree of contamination (Fig. 3, *b*).

The Pollution Load Index (PLI):

According to PLI, industrial 1 and 4 were highly polluted, while Industrial 2, 3 and control sites were moderately polluted to unpolluted (Fig. 3, *b*).

Pollution Index (PI):

According to PI indices, all studied sites were heavily polluted (Fig. 3, *b*).

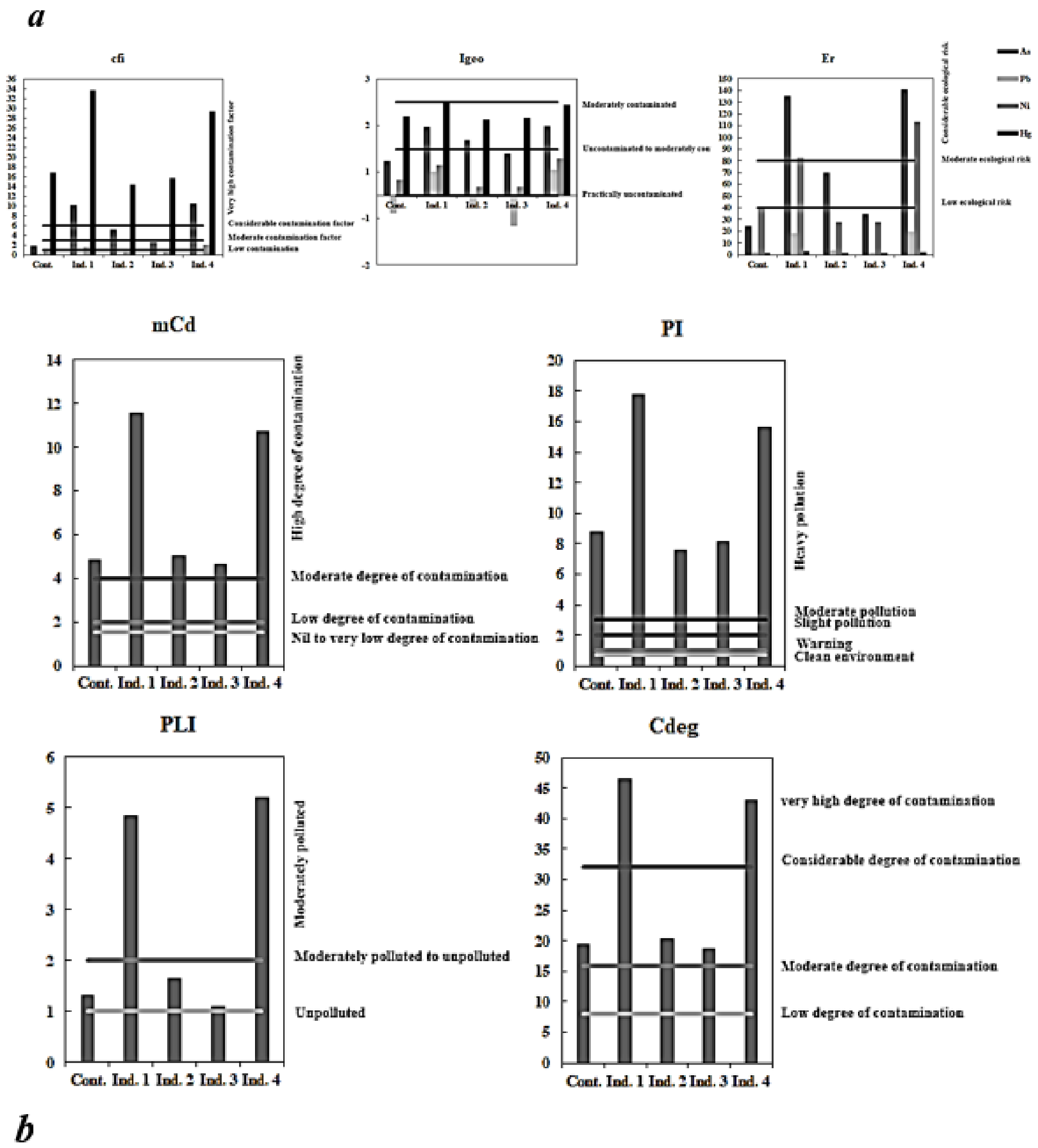


Fig. 3: Pollution indices calculated for soil's heavy metal at studied sites, (*a)*. Cf, Igeo, Er (*b)*. mcd, Pi, PLI, Cdeg**.**

Heavy Metal Contamination on Floral Coverage:

Soil heavy metals show a direct relation to floral coverage, as shown in Figure (4); the effect of heavy metals followed the following order Ni>Pb>As>Hg. This reflects the present observation of floral coverage, with AS the highest effective heavy metal alters and the floral coverage's role in polluted stations.

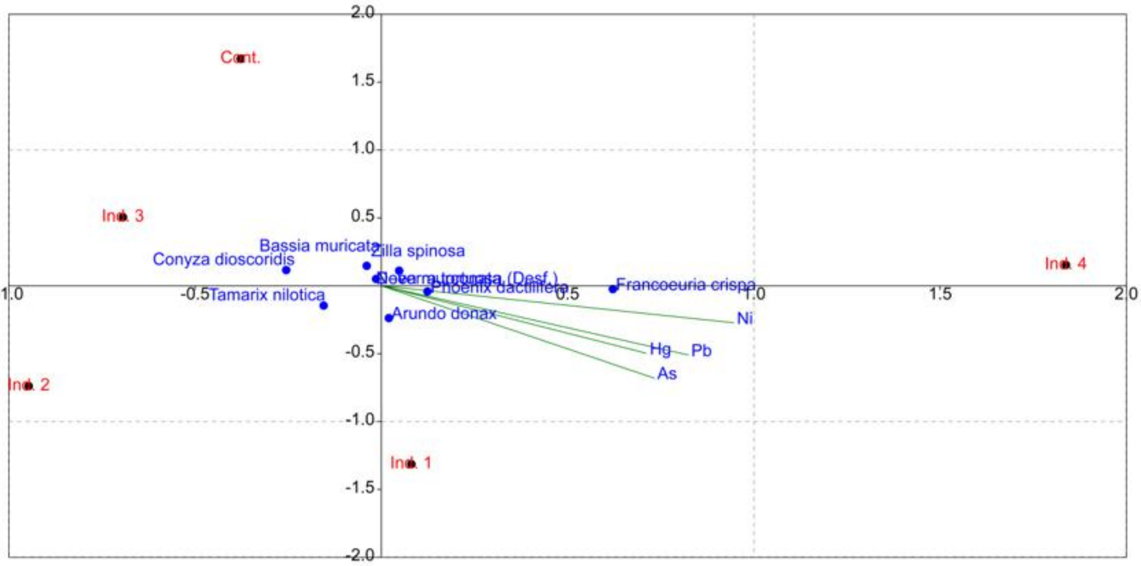


Fig (4): CCA represents soil heavy metal's role in floral distribution and coverage.

Enzymatic Activity:

Enzymatic activity of (GST and AChE) for *C. savignyi* have been observed as a biomarker for soil contamination at investigated sites, which reveals a significant seasonal variation ($P< 0.05$) over the period of the study for both enzymes; this change could be a direct effect of heavy soil metals (Table 4 & Fig. 5). The Enzymatic activity of (GST and AChE) of *C. savignyi* were rolled by the observed soil contamination as following equations:

$$GST\ (U/L) = 5973 - 38.81\,As + 640.0\,Pb - 54.05\,Ni - 2763\,Hg$$

$$AChE\ (U/L) = -72.20 + 1.253\,As - 15.62\,Pb + 1.088\,Ni + 55.38\,Hg$$

Table 4: GST and AChE enzymatic activities recorded for *C. savignyi* at investigated sites during the period of the study.

Enzymes	Site	Spring	Summer	Autumn	Winter
GST (U/L)	**Cont.**	474 ± 4.89^{b}	765 ± 7.34^{a}	648 ± 5.71^{c}	756 ± 4.89^{a}
	Ind. 1	212 ± 3.26^{c}	614 ± 4.08^{b}	703 ± 4.08^{a}	623 ± 4.08^{b}
	Ind. 2	851 ± 7.34^{a}	962 ± 8.16^{b}	429 ± 2.44^{c}	772 ± 4.89^{d}
	Ind. 3	225 ± 2.44^{c}	684 ± 5.71^{a}	673 ± 5.71^{a}	392 ± 3.26^{b}
	Ind. 4	372 ± 3.26^{a}	541 ± 4.89^{b}	396 ± 4.08^{c}	592 ± 4.08^{d}
AChE (U/L)	**Cont.**	38.7 ± 1.63^{b}	47.3 ± 2.28^{a}	36.8 ± 1.87^{b}	35.4 ± 1.55^{b}
	Ind. 1	51.4 ± 2.2^{a}	58.21 ± 2.77^{a}	51.75 ± 2.61^{a}	39.96 ± 1.63^{b}
	Ind. 2	53.88 ± 1.79^{b}	62.57 ± 1.79^{a}	57.73 ± 2.93^{ab}	51.53 ± 1.87^{b}
	Ind. 3	48.09 ± 1.87^{b}	56.92 ± 1.87^{a}	50.12 ± 1.55^{b}	47.53 ± 1.63^{b}
	Ind. 4	42.72 ± 1.46^{a}	68.9 ± 2.93^{b}	59.6 ± 2.77^{c}	31.68 ± 1.06^{d}

* *Means in the same row that do not share a letter differ significantly.*

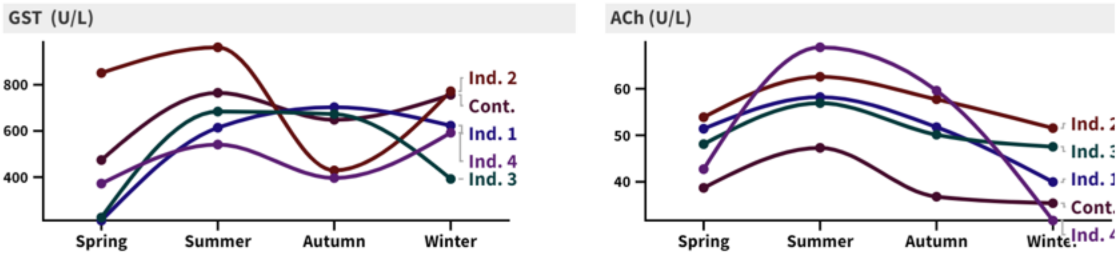


Fig. (5): GST and AChE enzymatic activities recorded for *C. savignyi* at investigated sites during the period of the study.

DISCUSSION

Climate change directly effects species survival, reproduction, and development (Bale *et al*., 2002). Nine plant species were identified at different study sites in the October district. *Zilla spinosa* (Family: Brassicaceae), *Tamarix nilotica* (Family: Tamaricaceae) and *Conyza dioscoridis* (Family: Compositae) are the most recorded species in study sites. In special, *Zilla spinosa* was the dominant species in all vegetation cover at different study sites, which agrees with Bream *et al. (*2019). Due to their large biomass, rapid growth, and capacity to adapt to extreme climates in nature, native vegetation on mining sites should be used as much as possible as a better indicator of reclamation effectiveness (Bandyopadhyay, 2022). Heavy metals in soil function as micronutrients; however, larger quantities hinder or slow plant development and metabolic functions (Dankoub *et al*., 2012; Taghipour *et al*., 2011). Some plants can tolerate elevated levels of heavy metals depending on the genetic imprint and genotype of the plant (Leitenmaier and Küpper, 2013, Pulford and Watson, 2003, Jamal *et al*., 2006).

Heavy metals are widely regarded as the most dangerous of all anthropogenic environmental contaminants due to their toxicity, persistence in soil and the environment, and contribution to the load of metal pollutants in terrestrial and aquatic food chains. Also, it directly affects ecological systems' resilience (Guo *et al*., 2012; Opaluwa *et al*., 2012; Gall *et al*., 2015). Therefore, the accumulation of heavy metals in soil was detected in the current study sites.

The present investigation of heavy metals in soil revealed the following accumulation order from high to low concentrations: As > Ni> Pb > Hg. Increased heavy metal concentration was seen at industrial 4 and 1 sites. This impact is more related to increased heavy metal emissions in heavy and medium areas than others. Bream *et al.* (2019) stated the increase in metals is correlated with industrial pollution sources. Anthropogenic activities are the primary source of soil arsenic (Bohn *et al.*, 1979). According to Bradl *et al.* (2005), Clay soils have larger levels of oxide minerals, which are excellent as adsorbers. Ore production and processing, galvanizing, domestic waste disposal, glassware manufacture, chemicals used for dyes, colors, wood preservatives, insecticides, pyrotechnics, cotton drying agents, oil and dissolvent recycling, and medicinal operations are the most common industrial uses of arsenic (Matschullat 2000). Ni levels in surface soils indicate soil formation and contamination. The major anthropogenic sources of Ni include alloys, chemical industries, petroleum refining, batteries, waste disposal, sewage sludge, fertilizer, transportation, fuel, and coal combustion (Reimann and De Caritat 1998). Pb concentrations in our environment have significantly grown and are gradually accumulating in the surface soil layers where man has utilized it for over 5,000 years (Bradl *et al.*, 2005). It is utilized in batteries, pigments, plastic stabilizers, ammunition, special alloys, pipes, and solder; its organic derivatives are employed in insecticides and as an antiknock agent in lead gasoline. (Reimann and De Caritat 1998). In contrast, the concentration of Hg measured in this investigation increased at industrial 1 and 4 as compared to the control site. Anthropogenic sources of Hg include the manufacturing of cement, the chemical and pharmaceutical industries, the burning of coal, and the incineration of municipal solid waste. Other potential indoor sources include building materials (furniture, paint, and fluorescent lamps), appliances, and electronics, including LCD displays, monitors, batteries, clothes dryers, irons, washing machines, fluorescent bulbs, neon lights, and thermometers. (Dahmardeh *et al*, 2022).

Contamination factors for heavy metals are in the order; Hg > As > Ni > Pb. Hg at all sites exhibits a high contamination factor. For As, industrial 1 and 4 represent a very high contamination factor, while Industrial 2 shows a considerable contamination factor, and industrial 3 and the control site revealed a moderate contamination factor. Finally, Pb and Ni in all sites show low contamination except industrial 1 and 4, exhibiting a moderate contamination factor. The calculated values of Cdeg vary from 18.77 to 46.44 in soil samples, where Cdeg at industrial 1 and 4 were at a very high degree of contamination, while industrial 2, 3 and the control site were at a considerable degree of contamination. Igeo was estimated for all metals using the background values established by (Taylor and McLennan,1995). Since there are no background values of these elements established in this city, The geo-accumulation Index (Igeo) for studied heavy metals displayed the following order: Hg > As > Pb > Ni in the soil samples. The risk factor was successfully applied to evaluate heavy metal contamination of soils in the environment. The Er values for nickel demonstrate a moderate ecological risk from this metal for all sites except industrial 4 considerable ecological risk. For arsenic, indicating a moderate ecological risk for all sites except industrial 1 and 4 considerable ecological risk. Lead and mercury values indicate a low ecological risk. Industrials 1 and 4 represent a very high degree of contamination. The PLI values indicate that industrial 1 and 4 were highly polluted.

Different xenobiotics and heavy metals were reacted to by changes in detoxification enzyme activity via metabolism, degradation, or even antioxidative activity to preserve vital body organs or molecules (Wilczek *et al*., 2008). As a result, the activity of detoxifying enzymes has been widely employed in monitoring environmental pollution as an early sensitive, responsive instrument and efficient biochemical biomarker (Praet *et al*., 2014; Bream *et al*., 2019). The maximum levels of all metals, As, Pb, Ni, and Hg, were found in the soil at Industrial 1 and 4 compared to the control site in this study. In different study sites, the activity of AChE and GST in *C. saviginyi*, and the results revealed a reduction in GST levels at the industrial sites (1 and 4) as compared to the control site. This result agrees with Sun *et al.* (2016), who established that GST activity reduced with an increase in the HM in the beetle's body burdens while it increases with the enhancement of xenobiotics. Also, Migula *et al.* (2004) proved that Correlations between GST activity and HM body burdens were shown to be positive with Cu in *G. stercorosus* and *S. caesareus*, and negative with Zn in *P. oblongopunctatus* and Cd in *S. caesareus*. The current results contradicted Stone *et al*. (2002), who found no significant variation in GST activity in male ground beetles collected from metal-polluted sites. On the other hand, our results reveal that AChE activity is higher in industrial areas than in control, and a high AchE level reflects an organism's ability to hydrolyze accessible acetylcholine and withstand the damaging effects of metals. Acetylcholinesterase (AChE) plays a vital role in neurotransmitters in vertebrates and invertebrates, responsible for the hydrolysis of acetylcholine to choline and acetic acid at cholinergic synapses and neuromuscular junction (Peña-Liopis *et al*., 2003). This is similar to Bream *et al.* (2019), who observed increases in the activity of AChE in industrial sites at El-Sadat City, Egypt. Also, Zatta *et al.* (2002) AChE activity was shown to be enhanced in rats given Alumine orally. Conversely, Lavado *et al.* (2006) reported that AChE was strongly inhibited in the muscle of several invertebrates collected from rivers contaminated with organic phosphates, carbamates, and heavy metals. Also, Aziz and Butt (2020) confirmed that in both spider species, AChE levels increased considerably with increasing Cu concentration but declined with increasing Pb deposition. Due to spider species' high metal body burden, combined Cu and Pb exposure reduced AChE activity significantly.

CONCLUSION

In conclusion, this work is the first to our knowledge to describe *C. saviginyi* ant as a bioindicator for industrial pollution on 6th October city, Egypt. Insects could be employed as heavy metal contamination biomarkers. Using insects as bioindicators provides a valuable tool for understanding the effects of climate change and industrial pollution on the environment. Insects, as sensitive organisms, can function as early warning signs of environmental changes. Nine plant species were identified at investigated study sites in the October district, with *Tamarix nilotica* as the dominant species in all vegetation cover at different study sites. The heavy metal accumulation in the soil was detected in the current study sites, and it was ordered as follows: As > Ni > Pb > Hg, with heavy metal content being higher in industrial sites compared to the control site. Pollution indices were calculated in the study sites, with Cf and Igeo indices highlighting mineral contamination and being ordered as Hg > As > Ni > Pb from high to low. Calculated Cdeg and PLI for industrial sites 1 and 4 revealed a very high degree of contamination, which can be attributed to the increase in industrial activity resulting from the chemical, Ceramic, Paints, and metallic industries that characterize this region. In contrast, the control site had a considerable degree of contamination. The current results highlight the inhibition of GST levels in C. saviginyi at the industrial site compared to the control site. In contrast, AChE activity increased in the industrial site compared to the control site, which might be due to heavy metals enhancing acetylcholine activity at synapses. Thus, antioxidant enzymatic activities serve as useful biomarkers for assessing and monitoring environmental contamination.

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