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Detoxification Enzymes of *Cataglyphis saviginyi* as a Biomarker for Underground Water Pollution at Kafr El-Zayat City, Gharbia Governorate, Egypt

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ABSTRACT

Underground water pollution has gained a growing concern in many communities worldwide. Flora and insects are crucial to our ecosystem, and their decline or depletion can have far-reaching consequences. The current study was conducted to investigate the environmental impact of industrial activities on underground water and floral coverage within the major industrial district of Kafr El-Zayat City, Gharbia Governorate, Egypt. Additionally, it aimed to evaluate the detoxification enzymatic activity of *Cataglyphis saviginyi* as a pollution biomarker. To achieve these objectives, water samples were collected from the investigated sites to assess heavy metal concentrations. Furthermore, a seasonal specimen of *C. saviginyi* was collected to investigate the enzymatic activity of acetylcholinesterase (AChE) and glutathione-S-transferase (GST). The current results highlighted the inhibition of GST levels in *C. saviginyi* at the polluted sites in comparison to the control site. In contrast, AChE activity increased. Consequently, the antioxidant enzymatic activities proved their usefulness as biomarkers for assessing and monitoring environmental contamination.

INTRODUCTION

Underground water pollution is a serious and growing concern for many communities around the world (**Goel, 2006**). Sources of pollution can vary from industrial activities to agricultural runoff, as well as from municipal sewage systems and poorly managed landfills. Once the underground water is contaminated, it can be challenging and expensive to remediate and may not be suitable for human consumption or other uses (**Muhammad & Zhonghua, 2014**). Underground water can contain heavy metals, harming human health and the environment (**Mahurpawar, 2015**). Heavy metals like lead, mercury and cadmium can leach into groundwater from surrounding soil and rock formations or industrial discharges and waste products (**Sankhla *et al.*, 2016)**. The presence of heavy metals in underground water can affect drinking water quality and threaten human health, especially in areas with a high concentration of heavy metals

(**Maleki & Jari, 2021)**. On the other hand, plants can absorb excessive amounts of heavy metals from the soil, which can subsequently become hazardous (**Monni *et al*., 2001**) when transmitted through the food chain (**Wuana & Okieimen, 2011; Opaluwa *et al*., 2012**). Plants are regarded as an "early warning" sign of stress symptoms caused by pollution. Heavy metals tend to accumulate in their tissues and fluids (**Białońska & Dayan, 2005**). Therefore, they appear to be extremely promising for eliminating toxins from the environment and might be used as bioindicators to detect the level of pollution (**Garbisu & Alkorta, 2003; United Nations Environment Programme (UNEP), 2010**).

Climate and land cover changes, such as cover, height, biomass, relative humidity, soil temperature, moisture, fertility and erosion; all have an impact on the structural features of plants in terrestrial ecosystems (**Ostberg *et al*., 2015)**. Human activities such as land cover changes; the introduction of exotic species, overexploitation, and pollution all have an impact on biodiversity reduction **(Pereira *et al*., 2012; Titeux *et al*., 2016; Zhu *et al*., 2019).** The use of biochemical indicators to assess organism's exposure to chemical pollution has been reviewed by **Min and Kang (2008)**, **Wilczek *et al*. (2008)** and **Van Praet *et al*. (2014**). They also have a wide range of enzyme activities that allow them to metabolize or break down various xenobiotics (**Wilczek *et al*., 2004; Augustyniak *et al*., 2005; Wilczek *et al*., 2008**). Furthermore, enzymes protect cells from oxidative damage, which suppresses antioxidant activity, hence protecting more critical molecules such as DNA, RNA and proteins (**Augustyniak & Migula, 2000**). GST enzyme is also considered an antioxidant, facilitating their conjugation with GSH, and producing non-toxic compounds (**Xu *et al*., 2015**). Acetylcholinesterase (AchE) is a neurotransmission hydrolase that facilitates the transmission of nerve impulses by hydrolytic acetylcholine into choline and acetate (**Kim & Lee, 2018**). Initially, it was used as a biomarker for numerous hazardous chemicals such as organophosphate and carbamate. However, its activity can also be affected by other xenobiotics such as heavy metals (**Van Praet *et al*., 2014; Bonnail *et al*., 2016**).

This study's main objectives were to assess underground water contaminated with heavy metals in Kafr El-Zayat City, Gharbia Governorate, Egypt and investigate ecological indicators to determine the impact of industrial emissions on vegetation coverage in the surrounding industrial areas. In addition, the activity of detoxifying enzymes in *Cataglyphis saviginyi* was detected as biochemical indicators and species-sensitive tools to pollution.

MATERIALS AND METHODS

Study area

Kafr El-Zayat is one of Egypt's major agricultural and industrial cities, with large fertilizer, pesticide and insecticide manufacturing plants. (**Abu Khatita & Bamousa, 2020**). It lies on the Rosetta branch of the Nile River. This area was chosen because it is considered one of the main industrial regions in Egypt, including several large factories, especially chemical manufacturers (**IPEN, 2006; Abdel-Halim *et al.*, 2013)**. The localities of the current study will divide polluted in comparison to natural or control sites into:

Cont. : Lies at N 30° 48' 29.1" - E 30° 52' 21.7" in Kafr Dima, Kafr El-Zayat, Gharbia Governorate, which is characterized by no human impact and natural habitat.

Pollute. (1): Lies at N 30° 49' 58.8" - E 30° 48' 24.5" at Banofar, Kafr El Zayat, Gharbia Governorate. This site represents the community of different types of factories such as *Salt and Soda* (produced oil, soap, wax, animal fodder, liquid, and solid sodium silicates) and El-Malyia (Pesticide Production Company, IFIC, produced superphosphate, different types of sulphuric acid, sodium fluorosilicate, ferrous sulfates).

Pollute. (2): Lies at N 30° 49' 58.8" - E 30° 48' 24.5" El Sharikat St, Kafr El Zayat, Gharbia Governorate. This site represents *El-Mobidat* (A pesticides and chemical company, KZ, produces more than 40 kinds of chemical products, including agricultural pesticides and household pesticides in addition to fertilizer).

Climatic factors

Climatic data were obtained from the meteorological station in EL-Gharbia Governorate, Egypt. The seasonal mean of average air temperature degree (^oC), Pressure (kPa), wind speed (m s^{-1}) and Rh (%) were the main climatic parameters.

Floral coverage

Identifying the plant species was 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

Heavy metals in the water samples estimated according to **APHA (1998)** were obtained from all study sites. 3 samples were collected from each site in clean plastic bottles for chemical analysis and analyzed by Varian vista AX CCD Simultaneous ICP-AES and expressed as ppm dry weight.

Biochemical analysis of enzymes

The importance of enzymatic biomarkers in detecting organisms' exposure to chemicals and environmental pollution is remarkable. They are typically more sensitive and represent early detectable responses to environmental changes (**Domingues *et al.*, 2010; Van Praet *et al*., 2014**). The current study advocated using specified enzymes such as 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 g of tissue insect on 1 ml saline solution 0.7 %) using a fine polytron homogenizer for 2min. The homogenates were then ice-centrifuged at 4000 rpm for 15min. 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 the Biodiagnostic Company, Egypt.

Glutathione-S-transferases

GST activity 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

Data were coded and entered using the statistical package SPSS V.22. Data were assessed for satisfying assumptions of parametric tests; continuous variables were subjected to Shapiro-Wilk and Kolmogorov-Smirnov test 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 for each group; post-hoc analysis was assessed, using Tukey pairwise comparison using MiniTab V 14; *P*-value was considered significant at<0.05. Regression was conducted to figure out the relation and the prediction equation between underground water heavy metals and *C. savignyi* detoxification enzyme; the analysis became available using SigmaPlot V 14.0. CCA was illustrated using Past V 4.12. Data were visualized when possible, using R studio V 2022.02.4.

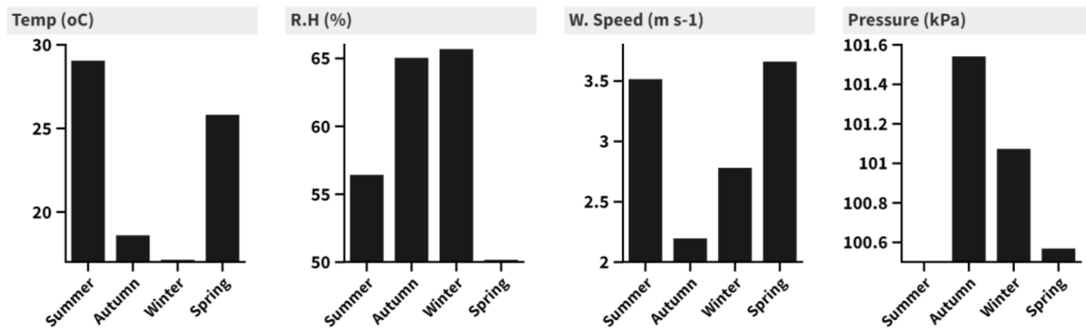
RESULTS

Climatic factor:

Fig. (1) shows the recorded meteorological data during the current investigation; there was a significant seasonal fluctuation in climatic parameters ($P < 0.05$). The highest temperature was 29.05 ± 0.15ºC during the summer of 2020. On the contrary, winter 2021 was characterized by its lower temperature (17.14 ± 0.26º C). Conversely, the greatest value of relative humidity was recorded during winter 2021(65.69 ± 0.14%), and minimum relative humidity (50.17 ± 0.97%) was recorded during spring 2021.

Furthermore, the highest value of wind speed was registered during spring 2021 (3.66±0.21ms-1), and the lowest value (2.2±0.10ms-1) was in autumn 2020. Meanwhile, the maximum value of pressure was recorded during autumn 2020 (100.54±0.45kpa), and the lowest value (100.50±0.29 kPa) was in the summer of 2020.

Fig. 1. Microclimatic factors throughout the current study period in Kafr El-Zayat City,



Gharbia Governorate, Egypt

Floral coverage

The diversity and fluctuation of species abundance characterize vegetation coverage. Ten plant species were identified (Fig. 2) at the investigated study sites in Kafr El-Zayat City. *Zilla spinosa* (Family: Brassicaceae) and *Tamarix nilotica* (Family: Tamaricaceae) are the most recorded species in study sites. In special, *Zilla spinosa* was the dominant species in all vegetation covered at different study sites. Its relative vegetation cover ranged between 25 & 50%. On the other hand, the Kafr El-Zayat Plant community was characterized by a variety of plant species (10 distinct species), with each of their relative vegetation ranging between 10 & 50%.

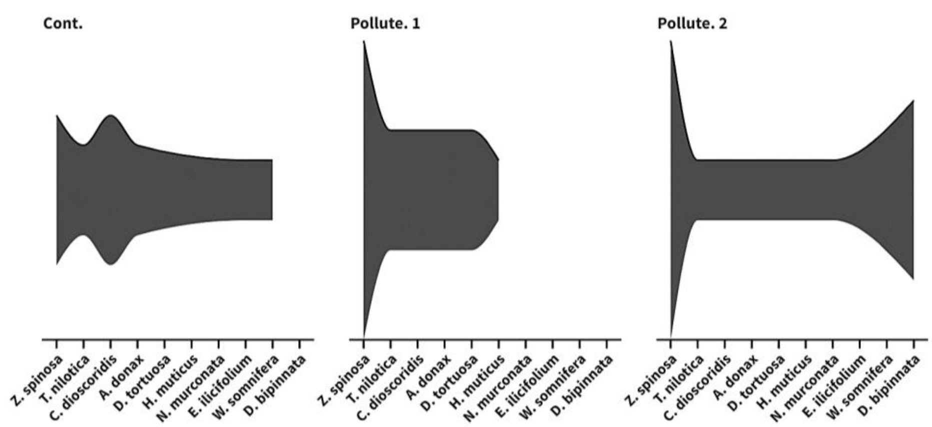


Fig. 2. Relative vegetation cover (value %) at different study sites in Kafr El-Zayat City, EL-Gharbia Governorate, Egypt

Heavy metal in water

Heavy metals in water such as arsenic (As), lead (Pb), nickel (Ni), and mercury (Hg) were measured at the investigated sites. All study sites in Kafr El-Zayat differed significantly (*P*< 0.05) from the control site regarding As, Pb, and Ni. Only Hg concentrations didn't vary significantly (*P*> 0.05) between study sites (Fig. 3).

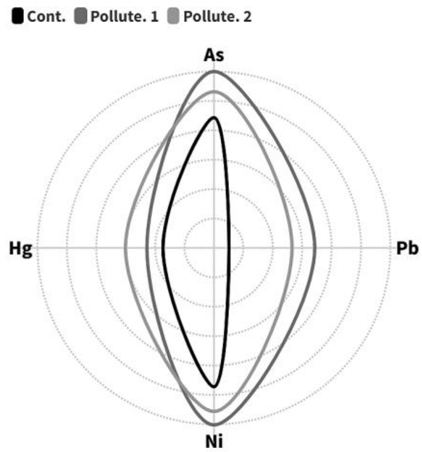


Fig. 3. Heavy metal contamination in water samples taken from studied sites throughout the current investigation in Kafr El-Zayat City, EL-Gharbia Governorate, Egypt

Heavy metal contamination on floral coverage

Water heavy metals show a direct relation to floral coverage, as shown in Fig. (4); the effect of heavy metals followed the order Ni> As> Pb> Hg; this reflects the present observation of floral coverage, proving that heavy metal alters the floral coverage in polluted stations.

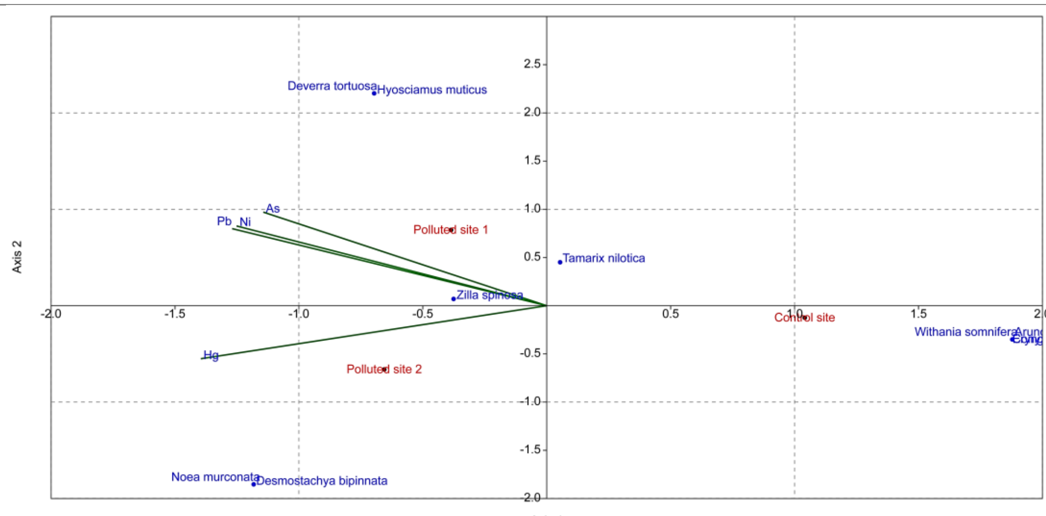


Fig. 4. CCA represents water-heavy metal's role in floral distribution and coverage

Enzymatic activity

Enzymatic activities of GST and AChE for *C. savignyi* was observed as a biomarker for water 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 metals (Table 1 & Fig. 5). The enzymatic activities of GST and AChE of *C. savignyi* were rolled by the observed water contamination as the following equations:

$$\boldsymbol{AChE}\ (\boldsymbol{U/L}) = 21.03 + 3.852\,As - 5.703\,Pb$$

$$\boldsymbol{GST}\ (\boldsymbol{U/L}) = 635.4 + 109.8\,As - 288.3\,Pb$$

Table 1. GST and AChE enzymatic activities recorded for *C. savignyi* at investigated sites during the study period

Enzyme	Site	Summer	Autumn	Winter	Spring
AChE(U/L)	Cont.	44.07±1.7[b]	40.05±0.35[c]	48.9±0.42[a]	40.9±0.56[b,c]
	Pollute. 1	57.8±0.56[a]	52.93±1.69[a,b]	56.18±1.52[a,b]	50.81±1.6[b]
	Pollute. 2	49.54±1.69[a,b]	43.06±1.8[b,c]	53.34±1.66[a]	38.6±1.62[c]
GST (U/L)	Cont.	1462±8.48[a]	980.5±12.02[c]	865.5±3.53[d]	1157.5±7.77[b]
	Pollute. 1	1072±9.89[a]	964±2.95[c]	845±5.66[d]	1031±8.48[b]
	Pollute. 2	1239±5.2[a]	849±2.82[b]	802±5.65[c]	805±5.8[c]

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

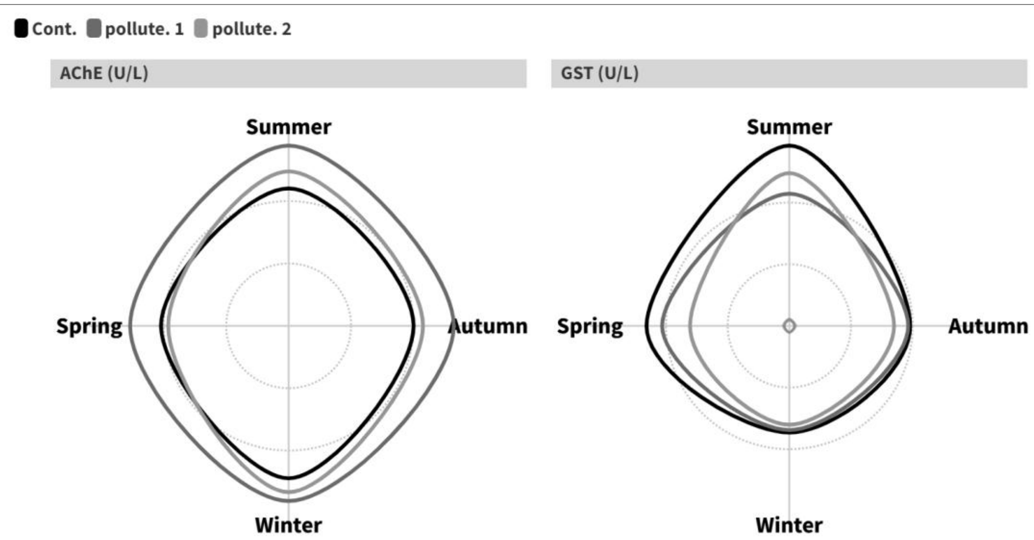


Fig. 5. GST and AChE enzymatic activities recorded for *C. savignyi* at investigated sites during the study period

DISCUSSION

Climate change, underground water pollution, flora and insects are all interconnected issues that ultimately significantly impact each other. Climate change is a phenomenon that has been accelerating at an alarming rate around the world, and it is causing havoc in many ecosystems. Increased temperatures, changing rainfall patterns and more extreme weather conditions are affecting the availability and quality of water resources, causing water pollution, and disturbing the natural habitat of flora and insects; climate change has a direct impact on species' survival, reproduction and development (**Bale *et al*., 2002**).

Ten plant species were identified at different study sites in the Kafr El-Zayat district. *Zilla spinosa* (Family: Brassicaceae) and *Tamarix nilotica* (Family: Tamaricaceae) are the most recorded species in the study sites. Remarkably, *Zilla spinosa* was the dominant species in all vegetation cover at different study sites, which agrees with the finding of **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)**. The flora has a significant role in maintaining biodiversity and ecosystems' health. Flora provides essential environmental services such as oxygen production, water regulation, nutrient cycling and carbon sequestration.

Water pollution is becoming a global concern as human population growth and industrialization increases. Industrial and agricultural pollution, plastic waste, and untreated wastewater all contribute to water bodies' contamination, ultimately affecting aquatic life and ecosystem services that freshwater ecosystems provide. In the results, the order of heavy metal accumulation detected in underground water in Kafr El-Zayat, was as follows: Ni > As > Pb > Hg.

Insects are vital in pollination, decomposition, and controlling other insect populations that could become pests. 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 (**Van Praet *et al*., 2014; Bream *et al*., 2019**). Our results estimated the activity of these two enzymes for *C. saviginyi*, and significant differences were found among different seasons. Seasonal differences in the AChE and GST activities were recorded, which might be related to the seasonal changes in water temperature (**Chitmanat *et al*., 2008**). This result agrees with that of **Bream *et al.* (2017)**. The present results revealed a reduction in GST levels at the polluted sites (1 and 2), as compared to the control site. This result coincides with that of **Sun *et al.* (2016)**, who established that GST activity reduced with the increase in the HM in the beetle's body burdens, while it increases with the enhancement of xenobiotics. This result is in conflict with that recorded in the study of **Bream *et al.* (2017)** who attributed the increased GST activity in selected aquatic insects to impacted environments. Moreover, **Migula *et al.* (2004)** proved the correlation between GST activity and HM body burdens. The current results contradicted that of **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 polluted 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. 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 the findings of **Bream *et al.* (2017)** and **Bream *et al.* (2019)** who observed increases in the activity of AChE in industrial sites at El-Sadat City, Egypt. Additionally, this agrees with that of **Gill *et al*. (1991)**, who reported increased AChE activity in skeletal muscles and brain of a fish species exposed to Cd for 48 hours. Additionally, **Zatta *et al.* (2002)** postulated that the AChE activity was 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. Furthermore, **Aziz and Butt, (2020**) confirmed that in both spider species, AchE levels increased considerably with increasing Cu concentration but declined with increasing Pb deposition.

CONCLUSION

In conclusion, it is crucial to take action to prevent contamination and ensure the sustainability of clean groundwater resources for future generations. This work is the first to describe *C.saviginyi* ant as a bioindicator for industrial pollution in Kafr El-Zayat City, Egypt. Insects could be employed as heavy metal contamination biomarkers. Using insects as bioindicators provides a valuable tool in 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. Ten plant species were identified at the investigated study sites in Kafr El-Zayat district, with *Zilla spinosa* being the dominant species in all vegetation cover at different study sites. The heavy metal accumulation in the water was detected in the current study sites, and it was ordered as follows: Ni > As > Pb > Hg, with heavy metal content being higher in polluted sites compared to the control site. The current results highlight the inhibition of GST levels in *C. saviginyi* at the polluted site, compared to the control site. In contrast, AChE activity increased, 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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