

Biochemical assessment of environmental pollution in the Alexandria governorate using bees and earthworms as bioindicators

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ABSTRACT

INTRODUCTION

Pollution represents a growing global challenge that threatens environmental sustainability, human health, and economic stability. It can be broadly defined as the introduction of harmful substances or forms of energy into ecosystems, manifesting in various types such as air, water, and soil contamination. According to the World Health Organization (WHO, 2021), air pollution alone accounts for approximately seven million premature deaths each year. The Lancet Commission on Pollution and Health identified air pollution as the fourth-leading risk factor for global mortality, exceeding the combined deaths from HIV, malaria, and tuberculosis (Fuller et al., 2022). Major sources of airborne pollutants include fossil fuel combustion, industrial emissions, and vehicle exhausts, which release harmful compounds such as particulate matter, nitrogen oxides (NO_x), and sulfur dioxide (SO₂). Chronic exposure to these pollutants has been strongly associated with respiratory diseases, cardiovascular disorders, and neurological impairments (Landrigan et al., 2022).

Both aquatic and terrestrial ecosystems are adversely affected by the accumulation of contaminants, including heavy metals, pesticide residues, and untreated wastewater. Approximately 80% of global wastewater is discharged untreated into

natural water bodies, posing severe threats to marine organisms and freshwater quality (UNESCO, 2021). In agriculture, the intensive application of pesticides and fertilizers has led to the degradation of nearly one-third (33%) of arable land worldwide, thereby threatening food security (FAO, 2022) and contributing to reduced soil fertility and crop productivity (Tóth et al., 2016). The global economic losses associated with pollution are estimated at around \$4.6 trillion annually (World Bank, 2019). Vulnerable and marginalized populations, particularly children, bear a disproportionate burden of pollution exposure, perpetuating cycles of poverty and health disparities (Bullard, 2020). Prolonged exposure to pollutants such as pesticides has been linked to elevated risks of cancer, neurodevelopmental disorders, and endocrine disruption. Additionally, emerging pollutants such as microplastics and plastic additives including phthalates and bisphenol, can adsorb hydrophobic toxicants and act as vectors for toxin bio-transfer (Rochman et al., 2019). The recent detection of microplastics in human placental tissue has raised critical concerns regarding potential transgenerational toxic effects (Ragusa et al., 2021).

Various living organisms can act as biological indicators (bioindicators) due to their measurable physiological and biochemical responses to environmental

contaminants. Honeybees (*Apis mellifera*) and earthworms (*Lumbricus terrestris*) have proven particularly useful for environmental monitoring. Pollutants, especially synthetic pesticides and industrial chemical contaminants, exert profound and often irreversible impacts on living organisms, thereby altering ecosystems and endangering biodiversity. Such impacts threaten not only ecological balance but also public health.

The intensive application of pesticides has led to their bioaccumulation in non-target species and biomagnification through food chains. Neonicotinoid insecticides, for instance, have been shown to disrupt pollinator behavior, particularly in bees, thereby jeopardizing the pollination of approximately 75% of food crops (IPBES, 2016). Honeybees (*Apis mellifera*), due to their extensive foraging ranges and collection of nectar and pollen, serve as dynamic indicators of both airborne and waterborne pollutants. Monitoring mechanisms involve assessing pollutant accumulation in honey and wax (Zhou et al., 2018), evaluating behavioral impairments and examining alterations in immune responses (Rothman et al., 2020).

Chemical runoff from agricultural and industrial sources also heavily contaminates freshwater and marine systems, directly impacting aquatic life, particularly fish. In soil, pesticides and heavy metals degrade quality by disrupting the microbial diversity essential for nutrient cycling. Glyphosate, for example, interferes with enzymes in the shikimate pathway in bacteria and fungi, thereby reducing organic matter decomposition and compromising soil fertility (Zobiole et al., 2010). Earthworms, which play a vital role in soil aeration and structure, show 50% mortality at sublethal chlorpyrifos concentrations (Pisa et al., 2017),

negatively affecting plant growth and exacerbating food insecurity.

Earthworms, particularly *Lumbricus terrestris*, often referred to as “ecosystem engineers”, are critical for soil health due to their roles in aeration and organic matter decomposition. Their sensitivity to chemical pollutants makes them reliable bioindicators for soil contamination assessments. Earthworm-based monitoring typically includes direct toxicity effects (Zobiole et al., 2010), bioaccumulation measurements (Spurgeon et al., 2020), and behavioral response assessments. In one laboratory study, *Lumbricus terrestris*, exposed to glyphosate exhibited a 30% reduction in biomass and a 50% decrease in cocoon production at concentrations as low as 1 mg/kg over 28 days (Zobiole et al., 2010). In field research conducted across contaminated sites in UK, *Lumbricus terrestris* accumulated cadmium and lead at levels five to ten times higher than those found in uncontaminated soils, confirming their reliability as indicators of soil health (Spurgeon et al., 2020). Moreover, standardized avoidance tests revealed that earthworms actively migrate away from soils contaminated with chlorpyrifos. At a concentration of 0.1 mg/kg, 80% of individuals relocated to uncontaminated zones within 48 hours, demonstrating their potential as early-warning bioindicators (OECD, 2016).

In parallel with biological approaches, technological advancements have led to the growing integration of environmental sensors for monitoring purposes. These devices are rapidly evolving and are being applied across multiple environmental contexts (Vielberth et al., 2020), suggesting that future environmental assessments will benefit from a hybrid framework combining bioindicators with sensor-based technologies.

This study aims to assess the extent and spatial distribution of environmental pollution in geographically representative areas of Alexandria Governorate through the application of biomonitoring techniques. By employing selected bioindicators and living warning systems specifically bees (as air bioindicators) and earthworms (as soil bioindicators), along with four enzymes (AChE, ALP, GST, and CAT) as biomarkers, this research seeks to evaluate the effectiveness of these bioindicator species as early-warning tools for detecting environmental stress. The outcomes will contribute to improved environmental risk assessment and the development of sustainable pollution management strategies.

MATERIALS AND METHODS

Organisms

Honeybees (*Apis mellifera*)

Honeybees are considered ideal bioindicators due to their foraging behavior and ability to accumulate pollutants from the environment. The bee body, like other insects, consists of three main parts: the head, thorax, and abdomen, along with six legs and two pairs of wings.

Earthworms (*Lumbricus terrestris*)

Earthworms are small, segmented, invertebrate organisms that live in soil and are highly sensitive to various environmental conditions. This sensitivity makes them good biological indicators for soil health.

Collection sites

Both organisms (honeybees and earthworms) were collected from three locations representing the geographical scope of Alexandria Governorate. These areas differ in pollution sources, including highly industrialized and densely

populated urban areas with significant vehicle emissions, zones adjacent to agricultural fields with pesticide exposure, and coastal regions affected by industrial and maritime pollution.

The sampling locations (Figure 1) were Maamora, Bakos, and Borg El-Arab, representing the eastern, central, and western parts of the Governorate, respectively. Honeybee samples were collected directly from beehives in local apiaries, while earthworms were collected from garden soils. A total of 100 individuals per site were collected for each organism to ensure data reliability.

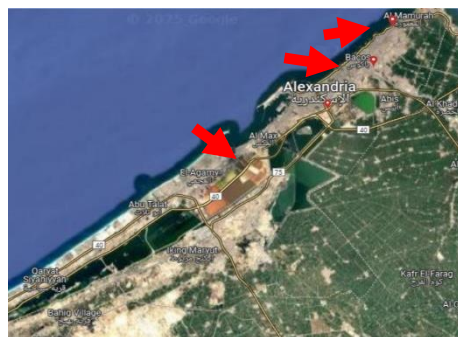


Figure 1. Picture showing sampling sites

Sample preparation

Organisms were anesthetized by cooling at 4°C, weighed, sacrificed, and dissected. For honeybees, head capsules were separated and homogenized in cold phosphate-buffered saline (PBS; 1:5 w/v) using a mortar and pestle. The homogenate was centrifuged at 6,000 xg for 15 minutes at 4°C, and the resulting supernatant was stored at -20°C for subsequent enzyme activity assays. For earthworms, coelomic fluid was gently extracted by applying light pressure, and tissues were homogenized in PBS. After centrifugation at 6,000 xg for 15 minutes at 4°C, the supernatant was collected and stored at -20°C until analysis.

Determination of Protein

Protein contents in the bee and earthworm supernatants were determined according to the method of Lowry et al. (1951). Five microliters of sample supernatant were added to 3 mL of Reagent C, and the mixture was incubated for 10 minutes at room temperature. Then, 0.3 mL of Reagent D was added and mixed thoroughly. After 20 minutes, the intensity of the developed blue color was measured at 750 nm against a blank. The protein concentration was calculated using a standard curve prepared from different concentrations of bovine serum albumin (BSA), where $K = 0.0094$.

Different reagents were prepared; Reagent A (20 g Na_2CO_3 and 0.5 g sodium potassium tartrate in 1000 mL of 0.1 N NaOH), Reagent B (0.5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 mL of a solution containing 1 g sodium potassium tartrate), Reagent C (freshly prepared by mixing 50 mL of Reagent A with 1 mL of Reagent B), and Reagent D (Folin–Ciocalteu’s phenol reagent diluted 1:1 with distilled water).

Enzyme activity assays

Acetylcholinesterase (AChE)

AChE activity was determined spectrophotometrically according to Ellman et al. (1961), using acetylthiocholine iodide (ATChI) as a substrate. In a typical assay, 50 μL of enzyme extract was added to 3 mL of a 1:1 mixture containing 2 mM substrate and 2 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). The developed yellow color was measured at 412 nm. A blank assay without enzyme served as the blank.

Alkaline Phosphatase (ALP)

ALP activity was determined using p-nitrophenyl disodium phosphate as the

substrate, and absorbance was measured at 405 nm.

Glutathione-S-Transferase (GST)

GST activity was measured using chloro-2,4-dinitrophenol (CDNB) as the substrate in the presence of glutathione (GSH). The reaction was monitored at 340 nm (Vessey and Boyer, 1984).

Catalase (CAT)

Catalase activity was assessed based on the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm, following the method of Song et al. (2009). This enzyme catalyzes the conversion of H_2O_2 into water and molecular oxygen.

The specific activity of each enzyme was calculated according to the equation:

$$\text{Specific activity} = \text{O.D} / (\text{mg protein} \times \text{min})$$

Statistical analysis

Enzyme activity data were analyzed using one-way ANOVA to determine significant differences among sites. Data are presented as mean $\pm$ standard deviation, and the results are illustrated using heat maps and bar charts.

RESULTS AND DISCUSSION

Protein concentrations in both bees and earthworms exhibited spatial variation among the studied sites in the Alexandria Governorate (Table 1). In bees, the highest mean protein concentration was detected at the eastern site ($0.117\% \pm 0.005$), followed by the middle site ($0.113\% \pm 0.004$), whereas the lowest value occurred at the Western site ($0.106\% \pm 0.007$). In earthworms, protein concentrations showed a similar spatial trend, with the highest level at the Middle site ($0.085\% \pm 0.005$), moderate at the Eastern site ($0.072\% \pm 0.011$), and the lowest at the Western site ($0.068\% \pm 0.004$). On

average, bees exhibited a higher overall protein concentration ($0.112\% \pm 0.005$) compared to earthworms ($0.075\% \pm 0.007$). The observed variations in protein concentrations among bees and earthworms from different sites in the Alexandria Governorate may reflect differences in environmental quality and exposure to pollutants. Generally, protein content is a sensitive biomarker of metabolic and physiological stress in organisms exposed to contaminants (Peakall, 2012; Van der Oost et al., 2003). The relatively higher protein levels in bees from the Eastern site and in earthworms from the Middle site could indicate favorable environmental conditions or lower pollutant stress, whereas the reduced protein concentrations at the Western site may suggest exposure to higher levels of environmental contaminants, such as heavy metals or pesticide residues, which are known to inhibit protein synthesis or increase protein degradation (Ivanina et al., 2008; Calisi et al., 2011). Similar findings have been reported in other bioindicator studies, where exposure to industrial and agricultural pollutants led to significant decreases in total protein content in both terrestrial and aquatic invertebrates (Javed and Usmani, 2019). Thus, the spatial variation observed in the present study suggests that protein concentration can serve as a useful biochemical indicator for assessing the impact of environmental pollution in the studied regions.

Table 1. Protein concentration (%) $\pm$ SD in bees and earthworm from different sites in the Alexandria Governorate

Sites	Protein concentration (%) $\pm$ SD	
	Bees	Earthworm
East	0.117 ± 0.005	0.072 ± 0.011
Middle	0.113 ± 0.004	0.085 ± 0.005
West	0.106 ± 0.007	0.068 ± 0.004
Average	0.112 ± 0.005	0.075 ± 0.007

The activity of the biomarker enzyme acetylcholinesterase (AChE) exhibited distinct variations between species and among the three sampling sites (East, Middle, and West) (Figure 2). In bees, AChE activity ranged from 1.688 ± 0.049 U/mg protein at the Middle site to 2.247 ± 0.047 U/mg protein at the West site, indicating site-dependent exposure to neurotoxic contaminants such as organophosphate and carbamate pesticides (Lionetto et al., 2013). In earthworms, AChE activity was consistently higher than in bees across all sites, ranging from 2.538 ± 0.151 U/mg protein at the Middle site to 4.265 ± 0.530 U/mg protein at the West site, likely reflecting their continuous contact with soil-bound neurotoxicants (Schreck et al., 2008).

Overall, the enzymatic biomarker responses revealed a clear spatial gradient of environmental stress, with the West site exhibiting the highest degree of biochemical perturbation. The observed site-dependent differences in enzyme activity highlight the usefulness of AChE and other enzymatic biomarkers in bees and earthworms as sensitive tools for assessing ecological risk and pollution exposure.

Figure 3 shows that, in bees, glutathione S-transferase (GST) activity increased from the East site (1.778 ± 0.078 U/mg protein) to the West site (2.539 ± 0.166 U/mg protein), reflecting enhanced xenobiotic metabolism (Furuhama et al., 2012). In earthworms, GST activity was also highest at the West site (3.623 ± 0.234 U/mg protein) and lowest at the East site (2.721 ± 0.418 U/mg protein), indicating pollutant-induced enzymatic induction (Hassan et al., 2016).

In bees, catalase (CAT) activity increased progressively from the East to

the West site, reaching a maximum value of 201.655 ± 13.149 U/mg protein, reflecting elevated oxidative stress in response to pollutant exposure (Vlahogianni et al., 2007). In earthworms, CAT activity was lowest at the East site (98.963 ± 15.209 U/mg protein) and peaked at the West site (193.361 ± 12.514 U/mg protein) (Figure 4), indicating pollutant-induced oxidative stress (Devi et al., 2024).

In bees, alkaline phosphatase (ALP) activity was highest at the Middle site (0.211 ± 0.019 U/mg protein) and lowest at the West site (0.160 ± 0.003 U/mg protein), suggesting alterations in cellular metabolism or membrane transport processes under sublethal stress (Mourete et al., 1991). In earthworms, ALP activity showed minimal variation across sites, ranging from 0.308 to 0.332 U/mg protein (Figure 5).

Figure 6 illustrates the average of enzyme activities in tested organisms from different sites in the Alexandria Governorate. Enzyme activity exhibited a clear spatial trend, increasing from the East site (59.0 U/mg) to the Middle site (92.3 U/mg) and reaching the highest level at the West site (102.1 U/mg). The differences among sites were statistically significant ($p < 0.05$), suggesting a gradient of biochemical response likely associated with varying degrees of environmental stress or pollutant exposure across the three locations.

In general, enzymatic variations across the three study sites revealed differential levels of environmental stress, likely driven by site-specific pesticide

applications, industrial emissions, and urban activities. The Western site exhibited the highest activities of acetylcholinesterase (AChE), glutathione S-transferase (GST), and catalase (CAT) in both bees and earthworms, indicating pronounced neurotoxic and oxidative stress responses. Elevated AChE activity is indicative of exposure to neuroactive pollutants, such as organophosphates and carbamates (von Wyl et al., 2023), whereas the concomitant increase in GST and CAT suggests enhanced detoxification and antioxidant defense mechanisms against reactive oxygen species (Gusti et al., 2021).

In contrast, the Middle site showed the highest alkaline phosphatase (ALP) activity and moderately elevated CAT levels, which may reflect sublethal effects on membrane transport and phosphatase-regulated metabolic pathways (Mourete et al., 1991). The Eastern site generally presented lower enzyme activities across all biomarkers, implying reduced exposure to environmental contaminants relative to the other sites.

The observed spatial gradient of enzymatic responses highlights the Western site as the most biochemically impacted, a pattern consistent with the higher industrial and vehicular emissions in western Alexandria. These results support the use of bees and earthworms as complementary bioindicators of terrestrial pollution, with earthworms responding more strongly due to direct soil contact, and bees reflecting airborne or floral contaminants (Chowdhury et al., 2023).

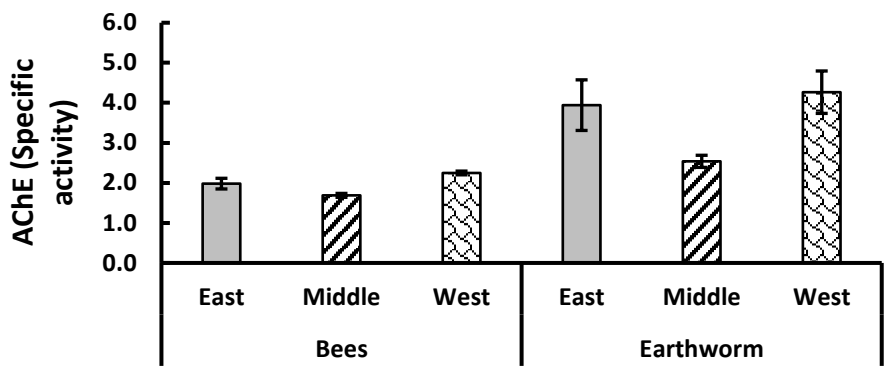


Figure 2. AChE Specific activity (U/mg protein) ± SD of bees and earthworms from different sites in the Alexandria Governorate.

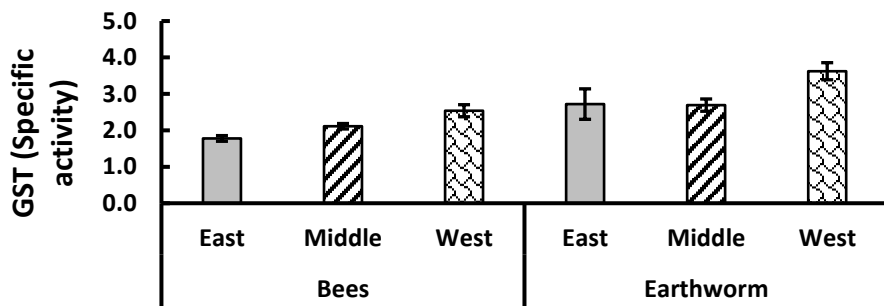


Figure 3. GST Specific activity (U/mg protein) ± SD of bees and earthworms from different sites in the Alexandria Governorate.

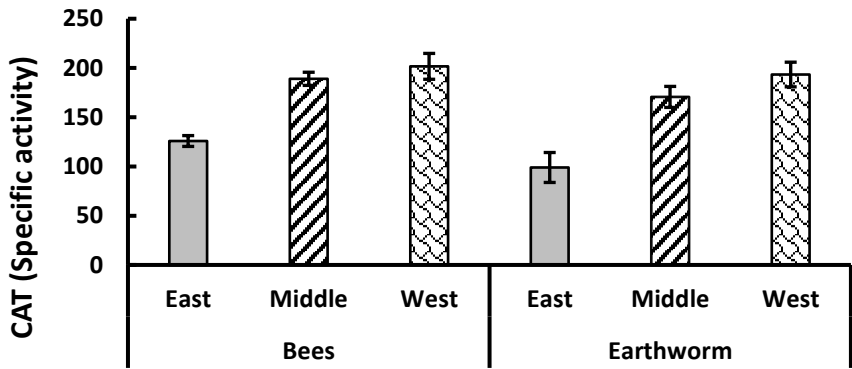


Figure 4. CAT Specific activity (U/mg protein) ± SD of bees and earthworms from different sites in the Alexandria Governorate.

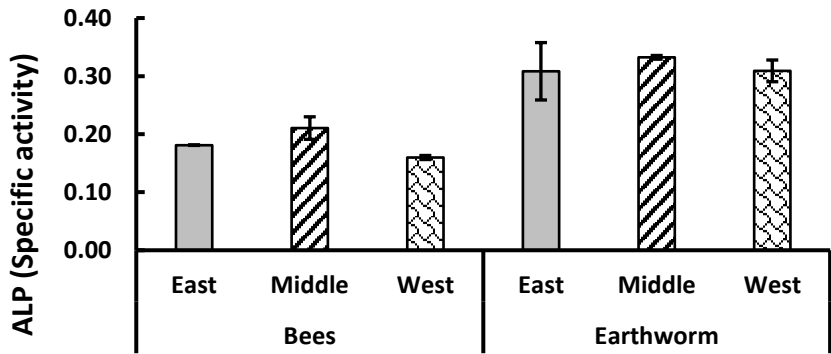


Figure 5. ALP Specific activity (U/mg protein) ± SD of bees and earthworms from different sites in the Alexandria Governorate.

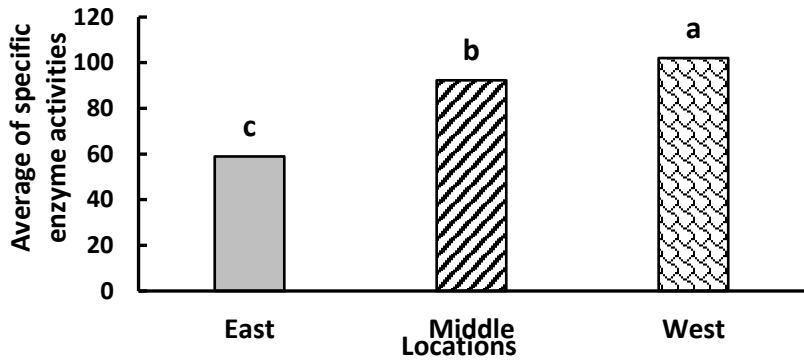


Figure 6. Average of specific enzymes activities (U/mg protein) of organisms from different sites in the Alexandria Governorate. Different letters above the bars indicate significant differences among means ($p < 0.05$).

The consistent elevation of AChE, GST, and CAT in both organisms at the Western site underscores the influence of anthropogenic stressors, including pesticides and industrial runoff, on ecosystem health (van der Oost et al., 2003; Jaffer et al., 2020).

Conclusion

The present study demonstrates clear spatial variations in protein concentrations and enzymatic biomarker activities in bees and earthworms across the Alexandria Governorate, reflecting differential environmental stress. Protein content was generally higher in bees than in earthworms, with reduced levels at the Western site suggesting greater exposure to pollutants. Enzymatic biomarkers, including acetylcholinesterase (AChE), glutathione S-transferase (GST), catalase (CAT), and alkaline phosphatase (ALP), exhibited a pronounced spatial gradient, with the Western site showing the highest biochemical perturbations. This pattern indicates heightened neurotoxic and oxidative stress associated with pesticide application, industrial emissions, and urban activities.

The Middle site displayed elevated ALP activity and moderate CAT levels, suggesting sublethal stress affecting membrane transport and metabolic regulation, whereas the Eastern site generally exhibited lower enzymatic activity, indicating comparatively lower contaminant exposure. The consistent elevation of AChE, GST, and CAT in both bees and earthworms at the Western site underscores the utility of these organisms as complementary bioindicators of terrestrial pollution, with earthworms reflecting soil-bound contaminants and bees providing insight into airborne or floral exposure.

Although the present investigation was limited by the number of samples collected, the consistent biochemical responses observed provided an early warning of pollution-related stress in the studied areas. These findings highlight the importance of continuous biomonitoring to identify pollution hotspots and to guide the development of effective management strategies aimed at reducing environmental contamination and protecting ecosystem health.

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التقييم الكيموحيوي للتلوث البيئي في محافظة الإسكندرية باستخدام النحل وديدان الأرض كمؤشرات حيوية

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يشكل التلوث البيئي تهديدًا كبيرًا لسلامة النظم البيئية، مما يستلزم استخدام مؤشرات حيوية حساسة للكشف المبكر والمراقبة المستمرة. هدفت هذه الدراسة إلى تقييم أنشطة أربعة من الإنزيمات الحيوية الهامة، وهي: أستيل كولين إستريز (AChE)، والفوسفاتاز القلوي (ALP)، وجلوتاثيون إس-ترانسفيراز (GST)، والكاتالاز (CAT)، بالإضافة إلى قياس تركيزات البروتين الكلي في نحل العسل (*Apis mellifera*) ودودة الأرض (*Lumbricus terrestris*) التي جُمعت من ثلاثة مواقع (الشرق، الأوسط، والغرب) داخل محافظة الإسكندرية، مصر. أظهرت النتائج وجود تباينات مكانية واضحة في نشاط الإنزيمات، حيث سجل الموقع الغربي أعلى مستويات من AChE و GST و CAT في كلا الكائنين، مما يشير إلى تعرض مرتفع للإجهاد العصبي التأكسدي الناتج عن الأراجح عن الأنشطة البشرية المكثفة، بما في ذلك استخدام المبيدات والانبعاثات الصناعية. أظهر نشاط إنزيم ALP تذبذبات طفيفة بين المواقع، وكان أقل استجابةً للإجهاد الناتج عن التلوث. بشكل عام، أبدت ديدان الأرض استجابات إنزيمية أقوى من نحل العسل، نتيجة لتلامسها المباشر مع التربة الملوثة، بينما عكس نحل العسل التلوث الجوي أو التلوث المرتبط بالنباتات المزهرة. توضح النتائج أن الجزء الغربي من محافظة الإسكندرية يعاني من أعلى مستويات الاضطراب الكيميائي الحيوي والعبء التلوثي. تؤكد هذه الدراسة أهمية الاستخدام المتكامل لكل من نحل العسل وديدان الأرض إلى جانب مجموعة من المؤشرات الإنزيمية كنهج فعال وشامل لتقييم التلوث الأرضي والمخاطر البيئية، مما يساهم في وضع استراتيجيات أكثر كفاءة لإدارة البيئة وحماية النظم البيئية. يمكن القول أنه بالرغم من أن الدراسة الحالية كانت محدودة بعدد العينات التي جُمعت، إلا أن الاستجابات البيوكيميائية المتسقة التي لوحظت وفُرت إنذارًا مبكرًا للإجهاد الناتج عن التلوث في المناطق المدروسة. فهذه النتائج تبرز أهمية الرصد الحيوي المستمر لتحديد بؤر التلوث الساخنة وتوجيه تطوير استراتيجيات إدارة فعالة تهدف إلى الحد من التلوث البيئي وحماية صحة النظام البيئي.