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Geospatial Biomonitoring of Pb and Cd Contamination in Bee Honey and Their Impact on Hydrogen Peroxide Activity

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Abstract— *Honey is globally revered as a complex bioactive matrix with potent therapeutic and antimicrobial virtues. However, its chemical integrity is increasingly threatened by escalating environmental degradation. This study aimed to evaluate the concentrations of cadmium (Cd) and lead (Pb) in honey samples taken from four environments: industrial, highway, agricultural, and rural, and to investigate their relationship to hydrogen peroxide (H_2O_2) production as an indicator of its antimicrobial activity. A clear pollution variation was observed, with honey samples from industrial environments recording the highest levels of cadmium (0.041 ± 0.004 mg/kg) and lead (0.12 ± 0.009 mg/kg), followed by honey samples from highway environments, and then agricultural environments. Rural honey recorded the lowest concentrations (cadmium: 0.010 ± 0.001 mg/kg; lead: 0.028 ± 0.003 mg/kg). Hydrogen peroxide (H_2O_2) production varied significantly with different levels of contamination and dilution ratios (25%, 50%, and 75%), peaking at a 50% dilution. Rural honey region exhibited the highest enzyme activity (42.8 ± 3.1 µg/g/h), while industrial honey region showed the lowest (28.6 ± 2.2 µg/g/h). A strong inverse correlation was found between heavy metal concentrations and hydrogen peroxide production, suggesting that cadmium and lead may inhibit glucose oxidase activity, thereby reducing honey's antimicrobial efficacy. These results highlight that honey from less polluted environments has superior biological properties, and emphasize the need for continuous monitoring of heavy metal pollution to ensure product safety and therapeutic quality.*

Keywords— Bee honey, Heavy metals, H_2O_2, Environmental pollution.

I. INTRODUCTION

Honeybees rely on four essential natural resources for their survival: water, resin, nectar, and pollen (Seeley, 2022). However, the concentration of heavy metals within honey is influenced by several variables, including the botanical origin of the flora visited, as well as prevailing ecological and climatic conditions (Clara et al., 2014). Furthermore, anthropogenic activities in the vicinity of apiaries play a decisive role in the elevation of metal concentrations in bee products (Bogdanov, 2006; Silici et al., 2016). In the contemporary discourse on environmental sustainability, Honey is considered an important bioindicator of environmental contamination due to the wide foraging activity of honeybees (Pohl, 2009; Bilandžić et al., 2019). The unique foraging ecology of *Apis mellifera*, covering vast areas of diverse flora, soil, and water, enables the honey matrix to function as a cumulative sentinel for environmental health (Bargańska et al., 2016; Pallottini et al., 2025). However, the chemical integrity of honey is increasingly compromised by the expansion of industrial hubs and high-traffic corridors, leading to significant fluctuations in (Anand, S., Deighton, M., 2019) Lead (Pb) and Cadmium (Cd) concentrations (Bogdanov, 2006; Staniškienė et al., 2006). While atmospheric Pb is primarily associated with industrial emissions and vehicular exhaust, Cd sequestration is frequently a byproduct of intensive agricultural practices and the persistent application of phosphate fertilizers (Al-Waili et al., 2025; Forster et al., 2023).

Beyond the immediate concerns of bioaccumulation, these heavy metals induce a profound and synergistic interference with the honey's redox-active matrix. The therapeutic value of honey is fundamentally anchored in its non-peroxide antimicrobial potency, driven by the enzymatic generation of hydrogen peroxide (H_2O_2) via the glucose oxidase pathway (White et al., 1965;

Brudzynski, 2020). Divalent ions like Pb^{2+} and Cd^{2+} act as aggressive non-competitive inhibitors; they exhibit a high affinity for the thiol groups (-SH) and active sites of the glucose oxidase protein (Sereia et al., 2017; Kędzierska-Matysek et al., 2018). This molecular binding triggers structural deformation and irreversible enzymatic denaturation, effectively silencing the honey's "oxidative shield" against bacterial pathogens. Furthermore, metallic contaminants often catalyze Fenton-like reactions, where the stable H_2O_2 reservoir is prematurely diverted into the formation of highly reactive hydroxyl radicals (•OH), depleting the natural antioxidant capacity and degrading bioactive polyphenols and flavonoids (Cianciosi et al., 2018).

The functional bioactivity of honey is profoundly dictated by the "dilution-activation" phenomenon. Under pristine conditions, glucose oxidase remains latent in concentrated honey and is triggered only upon aqueous dilution, leading to a characterized peak in H_2O_2 accumulation (Brudzynski, 2006; Bucekova et al., 2019). However, in samples characterized by high metallic loading, this critical oxidative flux becomes erratic; the "activation peak" is markedly blunted or entirely suppressed, rendering the honey biologically dysfunctional (Bucekova et al., 2023).

Consequently, this investigation seeks to bridge the gap between geospatial pollution data and the subsequent mechanistic disruption of enzymatic bioactivity. The primary objective is to evaluate how varying loads of Pb and Cd sourced from four distinct ecological terrains (industrial, high-traffic, agricultural, and rural) impair the dynamics of H_2O_2 production across a gradient of aqueous dilution ratios. By decoding the interplay between heavy metal contamination and enzymatic stability, this research aims to establish a robust toxicological foundation for more rigorous safety thresholds. Ultimately, this study provides a scientific framework to ensure the preservation of honey's therapeutic integrity and the bioactive properties within an increasingly polluted global landscape.

II. MATERIALS AND METHODS

2.1 Study Area and Experimental Design

The present study was conducted during the year 2026 to assess the environmental burden of heavy metal pollution in North Upper Egypt, specifically within the Beni-Suef Governorate. The research framework was designed to quantify the bioaccumulation of Lead (Pb) and Cadmium (Cd) in honey and to evaluate how such contamination modulates the antibacterial potency of the produced honey. To capture a representative environmental profile, four distinct ecological zones were categorized based on anthropogenic activity:

- **Industrial Zones:** Characterized by manufacturing activities
- **Highway Zones:** Regions adjacent to major highways and high-traffic roads
- **Agricultural Zones:** Areas dominated by intensive farming and phosphate fertilizer application
- **Rural Zones:** Serving as baseline or agro-ecosystem representations with minimal anthropogenic impact

Within each zone, five apiaries were selected via a stratified random sampling approach for specimen collection and subsequent laboratory examination.

2.2 Sample Collection and Matrix Preparation

Honey specimens were utilized as primary biomonitoring sentinels for Pb and Cd. In each ecological region, five representative apiaries were selected, with samples harvested from three independent colonies per apiary to ensure statistical robustness and account for intra-apiary variance.

2.3 Instrumental Determination of Heavy Metals

The quantification of Pb and Cd residues was performed using an Atomic Absorption Spectrophotometer (200 Series AA Systems). Following the analytical protocols established by Feldsine et al. (2002), the analysis was carried out at the specialized laboratories of the Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University. The metal concentrations in honey matrices were calibrated against certified standard solutions analyzed concurrently to validate accuracy. All analytical results were normalized and expressed in mg/kg.

2.4 Regulatory Framework and International Standards

To evaluate the toxicological significance of the detected levels, the data were benchmarked against the international maximum permissible limits (MPLs) dictated by the Codex Alimentarius, FAO, and WHO:

- **Lead (Pb):** The MPL in honey is strictly governed at 0.1 mg/kg. This threshold is critical given the neurotoxic profile of lead, particularly regarding pediatric health.
- **Cadmium (Cd):** While specifically tailored honey regulations are less frequent, a safety limit of 0.05 mg/kg was adopted based on general food safety paradigms. Cadmium is characterized by its high biological half-life, posing risks of renal dysfunction and osteological damage upon chronic exposure (Codex Alimentarius Commission, 2001; FAO/WHO, 2007; European Commission Regulation (EC) NO 1881/2006).

III. HYDROGEN PEROXIDE (H_2O_2) ANALYSIS

3.1 Sample Management and Dilution Protocols

Honey samples from the identified ecological zones were stored in sterile, hermetically sealed glass containers. To preserve the integrity of the light-sensitive glucose oxidase enzyme, samples were maintained at a controlled temperature of 25 ± 2°C in absolute darkness.

To investigate the kinetics of H_2O_2 generation, honey was reconstituted in sterile distilled water to yield three weight/volume (w/v) concentrations: 25%, 50%, and 75%. All dilutions were prepared immediately prior to the assay to mitigate enzymatic degradation.

3.2 Analytical Principle and Reagent Profile

The determination of H_2O_2 followed the methodology of Bogdanov et al. (2008), with optimized modifications. The assay hinges on the enzymatic oxidation of a chromogenic substrate; in the presence of peroxidase, H_2O_2 facilitates the formation of a colored complex, the intensity of which is proportional to the peroxide concentration.

The reagent system comprised:

- Endogenous Honey Glucose Oxidase
- Horseradish Peroxidase (HRP)
- Chromogenic substrate (o-dianisidine or ABTS)
- Phosphate buffer (stabilized at pH 6.5)
- Standardized H_2O_2 stock solutions

3.3 Assay Procedure and Spectrophotometric Quantification

A 1 mL aliquot of each honey dilution was reacted with 1 mL of phosphate buffer, 1 mL of chromogen, and 0.1 mL of peroxidase. The reaction mixture was incubated at 37°C for a duration of 30–60 minutes. The enzymatic reaction was quenched using sulfuric acid, and the resulting absorbance was recorded at 400–450 nm (optimized per the specific chromogen).

3.4 Calibration and Quality Assurance

A six-point standard curve was generated daily using known H_2O_2 concentrations (0–100 µg/mL). The final peroxide production rate was calculated and reported as µg H_2O_2 per gram of honey per hour.

To ensure the highest degree of analytical fidelity:

- All assays were performed in triplicate
- Method blanks (matrix-free) were processed alongside samples to account for reagent interference
- Standard curves were refreshed every 24 hours

3.5 Statistical Processing

Quantitative data are presented as Mean ± Standard Deviation (SD). Significant differences across geographical gradients and dilution factors were determined using one-way Analysis of Variance (ANOVA). Statistical significance was defined at a threshold of $P \leq 0.05$.

IV. RESULTS AND DISCUSSION

4.1 Spatial Heterogeneity of Heavy Metal Accumulation

The analytical results presented in Table 1 and Figure 1 reveal a significant spatial gradient ($P \leq 0.05$) in heavy metal concentrations across the studied regions. The highest levels of Lead (Pb 0.12 ± 0.009 mg/kg) and Cadmium (Cd 0.041 ± 0.004 mg/kg) were identified in the Industrial zone, followed by Highway and Agricultural zones. Conversely, Rural samples exhibited the lowest burdens (Pb 0.028 ± 0.003 mg/kg and Cd 0.010 ± 0.001 mg/kg).

The observed order (Industrial > Highway > Agricultural > Rural) directly reflects the intensity of anthropogenic pressure. In industrial corridors, atmospheric deposition of smelting by-products and fossil fuel combustion particulates settle on floral surfaces. Honeybees inadvertently transfer these metallic contaminants from nectar and pollen into the hive matrix.

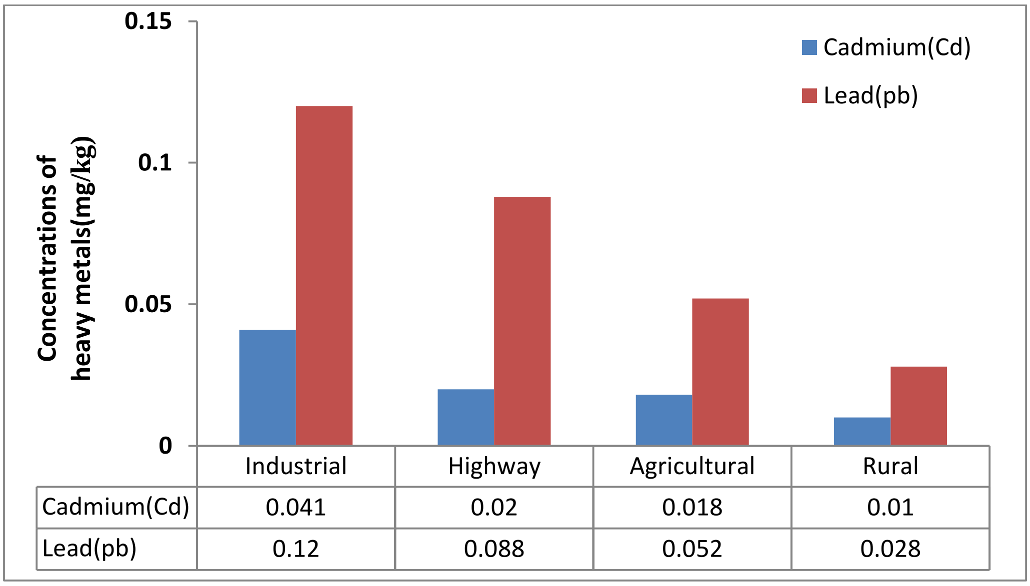
From the results obtained, Pb concentrations in industrial samples slightly exceeded the 0.1 mg/kg threshold established by the Codex Alimentarius (2001). Such levels are toxicologically significant as Pb is a non-essential element that disrupts cellular homeostasis and interferes with the sulfhydryl groups of vital proteins, posing long-term health risks to both honeybees and human consumers (Formicki et al., 2013; Perugini et al., 2011; Tuzen et al., 2007). The relatively low concentrations observed in rural samples confirm limited exposure to pollution sources, supporting the use of honey as a sensitive bioindicator of environmental contamination. Similar trends have been reported in previous studies, where urban and industrial honeys consistently showed higher metal burdens compared to rural counterparts (Solayman et al., 2016).

From a regulatory perspective, Pb levels in industrial samples approached or slightly exceeded the internationally recommended limit of 0.1 mg/kg (Codex Alimentarius, 2001), indicating potential health concerns. Lead is known to exert toxic effects through interaction with sulfhydryl groups in proteins, leading to enzyme inhibition and disruption of cellular functions (WHO, 2007). Cadmium, although present at lower levels, is also of concern due to its cumulative toxicity and long biological half-life.

TABLE 1
CONCENTRATIONS OF HEAVY METALS (mg/kg) IN HONEY FROM DIFFERENT REGIONS

Region	Cadmium (Cd)	Lead (Pb)
Industrial	0.041 ± 0.004^{a}	0.12 ± 0.009^{a}
Highway	0.020 ± 0.003^{b}	0.088 ± 0.006^{b}
Agricultural	0.018 ± 0.002^{c}	0.052 ± 0.005^{c}
Rural	0.010 ± 0.001^{d}	0.028 ± 0.003^{d}

Note: Different superscript letters within a column indicate significant differences ($P \leq 0.05$)



	Industrial	Highway	Agricultural	Rural
Cadmium(Cd)	0.041	0.02	0.018	0.01
Lead(pb)	0.12	0.088	0.052	0.028

FIGURE 1: Concentrations of Heavy Metals (mg/kg) in Honey by Region

4.2 Hydrogen Peroxide (H_2O_2) Production and Effect of Dilution

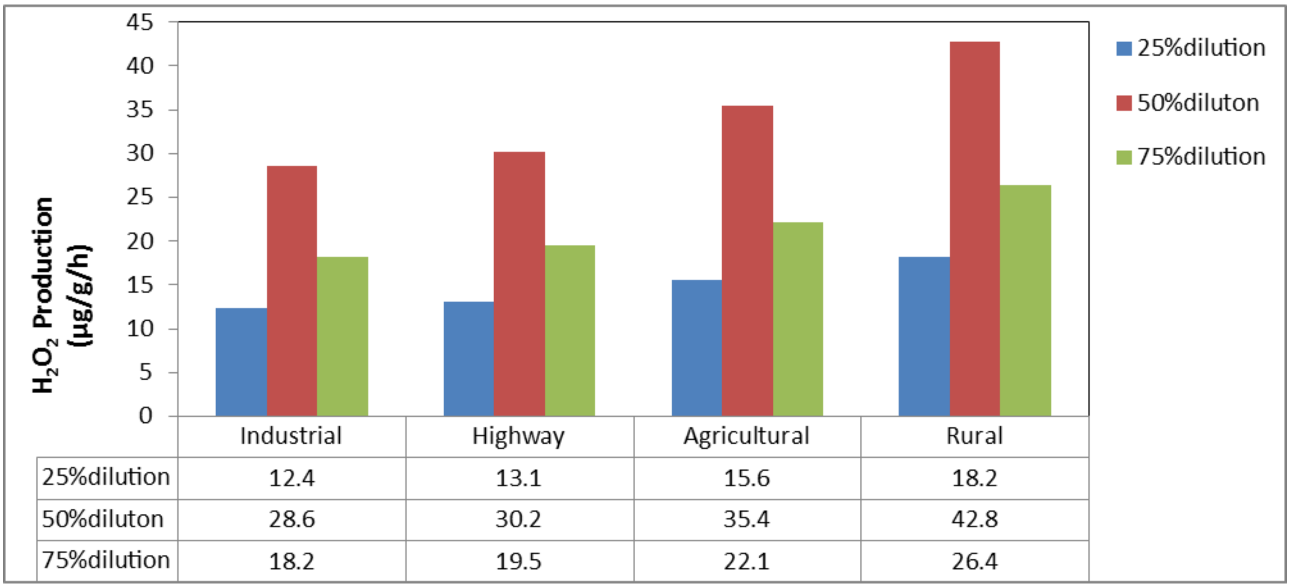
Hydrogen peroxide production (Table 2 and Figure 2) varied significantly among the studied samples and dilution levels. In all cases, H_2O_2 production increased with dilution up to 50%, followed by a decline at 75%. This trend clearly indicates that enzymatic activity is highly dependent on water availability. The highest H_2O_2 production was observed in rural honey samples (42.8 ± 3.1 µg/g/h at 50% dilution), whereas industrial samples exhibited the lowest activity (28.6 ± 2.2 µg/g/h at the same dilution level). This pattern suggests that environmental conditions not only influence contamination levels but also affect the functional properties of honey.

The increase in H_2O_2 production at moderate dilution can be explained by the activation of glucose oxidase, an enzyme that remains relatively inactive in undiluted honey due to low water activity and high osmotic pressure. Upon dilution, water availability enhances enzyme mobility and facilitates the oxidation of glucose to gluconic acid and hydrogen peroxide (Bogdanov et al., 2008; White, 1975). However, the observed decline at 75% dilution may be attributed to reduced enzyme concentration, low substrate (glucose) availability, and possible activity of catalase or other peroxide-degrading enzymes. This confirms that there is an optimal dilution level (50%) for maximum enzymatic activity (Brudzynski, 2006).

TABLE 2
H_2O_2 PRODUCTION (µg/g/h) AT DIFFERENT HONEY DILUTIONS

Region	25% Dilution	50% Dilution	75% Dilution
Industrial	12.4 ± 1.1	28.6 ± 2.2	18.2 ± 1.5
Highway	13.1 ± 1.2	30.2 ± 1.8	19.5 ± 1.4
Agricultural	15.6 ± 1.4	35.4 ± 2.5	22.1 ± 1.9
Rural	18.2 ± 1.5	42.8 ± 3.1	26.4 ± 2.2

Note: The bioactivity of honey is triggered by dilution, which activates glucose oxidase



	Industrial	Highway	Agricultural	Rural
25%dilution	12.4	13.1	15.6	18.2
50%diluton	28.6	30.2	35.4	42.8
75%dilution	18.2	19.5	22.1	26.4

FIGURE 2: H_2O_2 Production (µg/g/h) at Different Honey Dilutions

4.3 Impact of Environmental Contamination on Honey Bioactivity

A key finding of this study is the marked reduction in H_2O_2 production in honey samples from polluted environments. Industrial and highway samples consistently exhibited lower enzymatic activity compared to rural and agricultural samples. This reduction can be attributed to the inhibitory effects of heavy metals on enzyme systems. Both Cd and Pb have a strong affinity for functional groups in proteins, particularly sulfhydryl (-SH) groups, leading to conformational changes and loss of enzymatic activity. Additionally, heavy metals can induce oxidative stress, generating reactive oxygen species (ROS) that further damage proteins and enzymes (Estevinho et al., 2012; Brudzynski, 2006). Moreover, polluted environments may affect floral diversity and nectar composition, indirectly influencing the biochemical profile of honey. Rural areas, characterized by lower pollution and higher botanical diversity, provide more favorable conditions for maintaining enzyme integrity and bioactive compounds.

4.4 Correlation between Heavy Metals and H_2O_2 Production

An inverse relationship was clearly observed between heavy metal concentrations and hydrogen peroxide production. Samples with higher levels of Cd and Pb showed significantly lower H_2O_2 production, particularly at the optimal dilution level (50%). This relationship can be mechanistically attributed to: heavy metals binding to active sites of glucose oxidase, reducing catalytic efficiency; structural changes decreasing enzyme stability and functionality; and metal-induced ROS leading to breakdown of enzymatic systems (Estevinho et al., 2012).

When compared with international standards, most honey samples fell within acceptable limits, except for slight exceedance or borderline levels of Pb in industrial regions. This highlights the importance of continuous monitoring, especially in areas with high anthropogenic activity. The results of this study are consistent with those reported by Perugini et al. (2011) and Tuzen et al. (2007), who documented higher heavy metal levels in urban and industrial honey samples. Similarly, the observed variation in H_2O_2 production aligns with previous studies emphasizing the role of dilution and environmental factors in modulating enzymatic activity (Bogdanov et al., 2008; Brudzynski, 2006).

V. CONCLUSION

The present study clearly demonstrates that environmental conditions play a crucial role in determining both the safety and functional quality of bee honey. Honey samples collected from industrial and highway regions exhibited elevated levels of heavy metals, particularly Pb and Cd, compared to those from agricultural and rural areas, reflecting the direct impact of anthropogenic pollution sources. In parallel, hydrogen peroxide (H_2O_2) production, which represents a key indicator of honey's antimicrobial activity, showed significant variation among samples and dilution levels. The highest enzymatic activity was consistently observed at 50% dilution, confirming the existence of an optimal dilution threshold for glucose oxidase activation. Notably, honey from less polluted environments demonstrated significantly higher H_2O_2 production, whereas samples with higher heavy metal contamination exhibited reduced bioactivity. The observed inverse relationship between heavy metal concentrations and hydrogen peroxide production highlights the detrimental effect of environmental contaminants on enzymatic systems in honey. This suggests that pollution not only compromises the chemical safety of honey but also diminishes its biological and therapeutic properties. Overall, the findings emphasize the dual role of honey as both a nutritious food product and a sensitive bioindicator of environmental pollution. Continuous monitoring of heavy metals in bee honey, along with evaluation of its functional properties, is essential to ensure product quality, protect public health, and support sustainable apicultural practices.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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