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RESEARCH PAPER

Sublethal Effects of Urban Air Pollution on Foraging Activity and Colony Health of the Asian Honey Bee (Apis Cerana) in Baghpat District, India

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Abstract

The study investigated the sublethal effects of air pollution on the Asian honey bee (Apis cerana) in Baghpat District, Uttar Pradesh, India. Honey bee colonies from an urban site characterized by high vehicular emissions and elevated particulate matter concentrations were compared with colonies from a less polluted rural area. A total of sixty worker bees were sampled, with thirty individuals collected from each location. Air quality was assessed using PM2.5 and PM10 measurements, while bee health was evaluated through hemocyte density analysis, foraging activity observations, and mortality assessment. The polluted site exhibited substantially higher particulate matter levels (PM2.5: 95 µg/m³, PM10: 180 µg/m³) compared with the rural site (PM2.5: 28 µg/m³, PM10: 60 µg/m³). Results demonstrated a significant decline in the mean hemocyte count of bees inhabiting polluted environments (4.8×10^6 cells/mL) relative to those from the rural site (7.3×10^6 cells/mL), indicating possible suppression of immune function. Foraging activity was also reduced, with urban bees performing an average of 58 trips per hour compared to 89 trips per hour in the rural population. Furthermore, mortality rates were markedly higher in the polluted area (14.5%) than in the rural environment (5.2%). Statistical analysis confirmed that these differences were significant ($p < 0.05$). The findings suggest that chronic exposure to urban air pollutants adversely affects the physiological condition, behavioral performance, and survival of Apis cerana. These impacts may ultimately reduce pollination efficiency and threaten colony sustainability. The study highlights the importance of improving air quality and implementing pollinator conservation strategies in rapidly urbanizing regions.

KEYWORDS:
Apis cerana, air pollution, particulate matter, hemocyte density, foraging behavior, pollinator health, colony survival, Baghpat.

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1. Introduction

Pollinating insects are fundamental components of terrestrial ecosystems because they facilitate plant reproduction and contribute directly to agricultural production. Among these insects, Apis cerana (Asian honey bee) is one of the most important pollinator species in India, supporting the productivity of numerous food crops, fruit trees, oilseed plants, and wild flowering vegetation. The ecological and economic value of honey bees has increased the need to understand the environmental factors that may threaten their health and survival.

Previous investigations have shown that deteriorating air quality can have harmful effects on honey bee populations. Negri and co-workers (2015) reported that honey bees readily collect and retain airborne particulate contaminants, demonstrating their value as indicators of environmental pollution. Research conducted by Khan et al. (2023) revealed that particulate pollution can disrupt normal bee activities, leading to reduced foraging performance and impaired colony development. Fuentes et al. (2016) reported that atmospheric pollutants degrade floral volatile compounds, reducing the distance over which floral scents can be

detected by pollinators and consequently increasing foraging times. Likewise, Dey et al. (2020) found that bees exposed to polluted conditions exhibited signs of physiological stress, including lower immune cell abundance, decreased visitation to flowers, and higher mortality rates. Thimmegowda et al. (2020) demonstrated that pollinators exposed to polluted environments exhibited reduced flower visitation rates, increased physiological stress, and altered behavioral patterns. Skuza et al. (2023) assessed environmental stress in genetically related honey bee foragers maintained under urban and rural conditions and found higher stress indicators among bees inhabiting urban environments. Furthermore, Zhao et al. (2022) demonstrated that environmental stress can modify immune functions in Apis cerana, potentially reducing the ability of bees to cope with pathogens and other challenges. Together, these findings indicate that air pollution is an important environmental stressor capable of compromising honey bee health, survival, and pollination efficiency. Wilson, Porter, and Carril (2024) estimated that millions of bees may be killed daily through vehicle collisions on road networks, emphasizing the cumulative effects of urbanization on pollinator decline.

Air pollution has emerged as a major environmental concern associated with rapid urban growth, industrialization, and increasing vehicular traffic. Urban atmospheres often contain elevated levels of particulate matter (PM2.5 and PM10), gaseous pollutants, and trace contaminants that can adversely affect living organisms. Honey bees interact continuously with the surrounding environment while collecting nectar, pollen, water, and plant resins. During these activities, they are exposed to airborne contaminants that may accumulate on their bodies or enter their physiological systems. Such exposure can interfere with sensory perception, reduce navigation efficiency, alter foraging patterns, and increase physiological stress. Long-term exposure to polluted environments may therefore compromise colony performance and reduce pollination services.

The immune system of insects serves as a critical defense mechanism against pathogens and environmental stressors. Hemocytes, the cellular components of insect hemolymph, are involved in immune responses such as phagocytosis, encapsulation, and wound repair. Variations in hemocyte density can provide valuable information regarding the physiological condition and immune competence of honey bees exposed to environmental challenges. Baghpat District of Uttar Pradesh contains both densely populated urban areas and relatively less polluted rural landscapes, creating an opportunity to investigate the biological consequences of differing air quality conditions. By comparing honey bee populations from these contrasting environments, it is possible to assess whether air pollution influences immune health, foraging activity, and colony survival.

2. Materials and Methods

The study was conducted to evaluate the effects of air pollution on the immune status and behavior of worker honey bees (Apis cerana) in District Baghpat, Uttar Pradesh. Two study sites were selected based on their pollution levels. The first site was located in an urban area exposed to heavy vehicular traffic and elevated particulate pollution, while the second site was situated in a rural agricultural region with relatively clean air and served as the control site. Healthy worker bees were collected from established colonies at both locations. A total of sixty worker bees were sampled, with thirty individuals obtained from each site. Sampling was carried out during similar weather conditions to minimize environmental variation. Air quality at each location was assessed by measuring particulate matter concentrations, specifically PM2.5 and PM10. These measurements were recorded during the sampling period to characterize the pollution status of each site.

To quantify hemocyte density in Apis cerana worker bees, foragers were collected from hive entrances between 10am and 12pm to standardize age and task. A minimum of 30 bees per treatment group was used to ensure statistical power. Bees were anesthetized in batches of five to six on ice for 5 to 7 minutes, and typically weigh 80 to 90mg. The abdominal surface of each bee was sterilized with 70% ethanol before hemolymph extraction. For hemolymph collection, each bee was placed dorsal-side up under a dissecting microscope. A 31G needle was inserted at a 45° angle between the third and fourth abdominal tergite to a depth of approximately 0.5mm. Gentle pressure was applied to the thorax, and 6 to 8 µL of hemolymph was collected with a 10µL pipette. The hemolymph was immediately transferred to a 1.5mL tube containing an equal volume of cold 1x PBS or Schneider's insect medium and mixed 1:1 to prevent melanization, as A. cerana hemolymph clots within 30 seconds. All samples were kept on ice during processing.

Total hemocyte counts were determined by mixing 10µL of diluted hemolymph with 10µL of 0.4% Trypan blue to assess viability. The mixture was loaded into a BioRad TC20 automated cell counter or a hemocytometer and counted within 3 minutes to avoid clumping. Total cells per mL, live cells per mL, and percent viability were recorded. When using a hemocytometer, cell density was calculated using the formula:

Cells/mL = (Average count per square × 10^4 × Dilution factor) / No. of squares counted

The dilution factor was 4 due to the sequential 1:1 dilutions with PBS and Trypan blue. For differential hemocyte counts, 5µL of diluted hemolymph was smeared on a clean slide and air dried for 30 minutes. Slides were fixed in 10% formalin for 30 minutes, rinsed with 1x PBS, and stained with 5% Giemsa for 20 minutes before a final rinse in distilled water and air drying. Under 40x magnification, 200 cells per slide were classified as prohemocytes, characterized by a small round shape and high nucleus to cytoplasm ratio, plasmatocytes with irregular spreading morphology, granulocytes containing distinct cytoplasmic granules, or oenocytoids and unidentified cells. Data were analyzed using Student's t-test for comparisons between two groups or ANOVA

for three or more groups, with significance set at $p < 0.05$. Healthy A. cerana foragers were expected to have a baseline hemocyte density of 5.0 - 7.5 × 10^6 cells/mL. Behavioral observations were conducted to assess the impact of pollution on bee activity. Foraging behavior was monitored during peak activity hours 08:00AM to 11:00 AM, and the number of foraging trips made by worker bees was recorded. Colony activity and mortality rates were also observed and documented throughout the study period. The collected data were analyzed statistically by calculating mean values and standard deviations. Differences between polluted and rural sites were evaluated using Student's t-test, and results were considered statistically significant when the probability value was less than 0.05.

3. Results

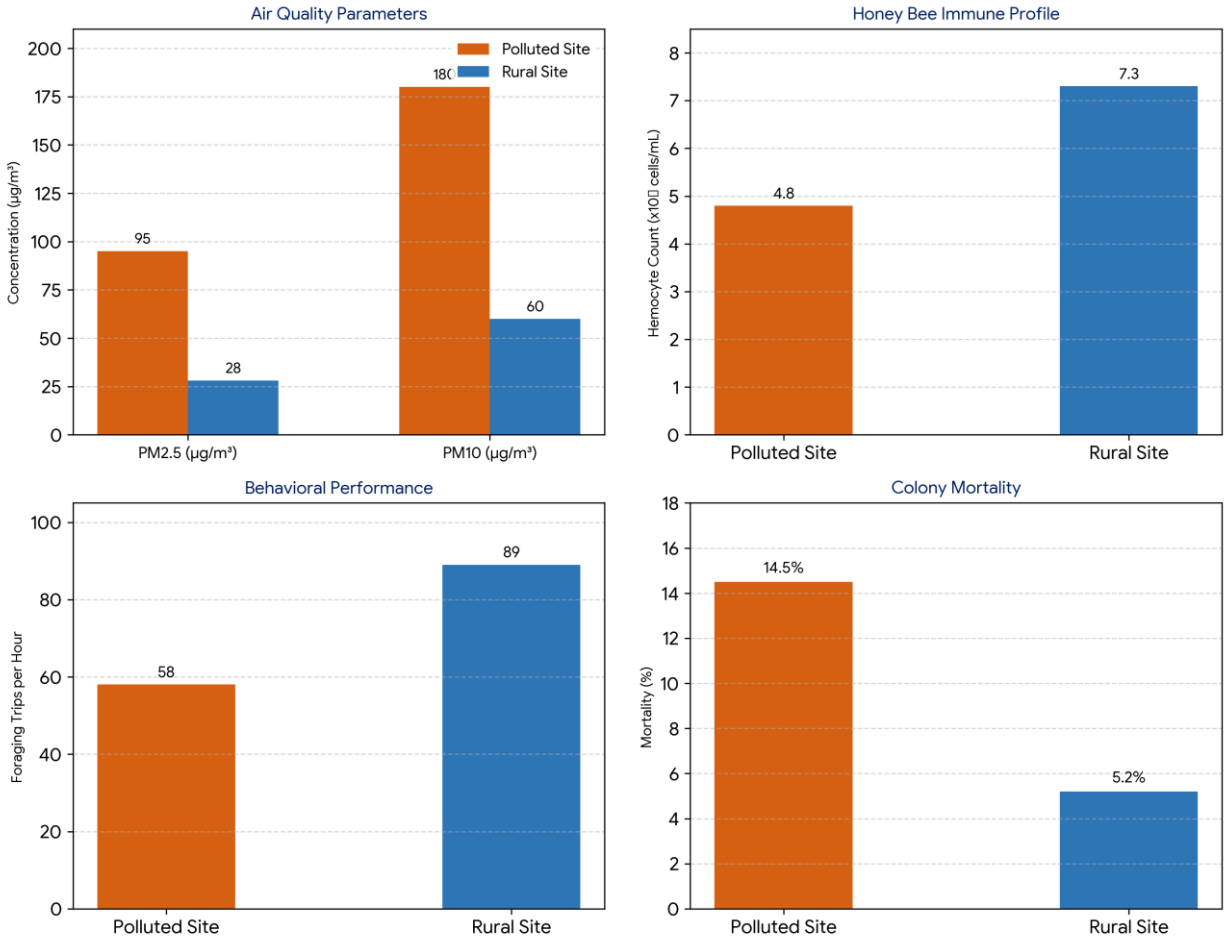
Air quality measurements showed clear differences between the two study sites. The polluted site recorded higher concentrations of particulate matter, with PM2.5 reaching 95 µg/m³ and PM10 reaching 180 µg/m³. In contrast, the rural site had much lower values of 28 µg/m³ for PM2.5 and 60 µg/m³ for PM10. The health status of Apis cerana worker bees was influenced by these environmental conditions. Bees collected from the polluted site had a lower average hemocyte count of 4.8 × 10^6 cells/mL, whereas bees from the rural site showed a higher hemocyte count of 7.3 × 10^6 cells/mL. This reduction suggests that exposure to elevated air pollution may negatively affect the immune system of honey bees.

Behavioral observations also revealed notable differences between the two group parameters. Worker bees in the rural area performed an average of 89 foraging trips per hour, while bees in the polluted area completed only 58 trips per hour. This decrease indicates that air pollution may interfere with normal foraging activity and reduce colony productivity. Mortality rates were also higher in the polluted environment. Bee mortality reached 14.5% at the polluted site compared with only 5.2% at the rural site. These findings indicate that colonies exposed to higher levels of air pollution experienced greater physiological stress and reduced survival. Statistical analysis using Student's t-test showed significant differences between the polluted and rural sites ($p < 0.05$) confirming that the observed changes were unlikely to have occurred by chance.

Effect of Air Pollution on Apis cerana: Air Quality, Immune Response and Behavior

Parameter	Polluted Site	Rural Site	Interpretation
PM2.5 (µg/m³)	95	28	Higher PM2.5 at polluted site
PM10 (µg/m³)	180	60	Higher PM10 at polluted site
Hemocyte Count (cells/mL)	4.8 × 10^6	7.3 × 10^6	Lower immune cells at polluted site
Foraging Trips / hr	58	89	Reduced foraging activity at polluted site
Mortality (%)	14.5	5.2	Higher mortality at polluted site

Comparative Analysis (Polluted Site vs Rural Site)



Statistical Analysis: Student's t-test was used to compare means between polluted and rural sites. Significance level: $p < 0.05$

4. Discussion

The results from Baghpat District show clear differences in Apis cerana health between polluted and rural environments. Bees from the urban site, where PM2.5 was 95 µg/m³ and PM10 was 180 µg/m³, had a mean hemocyte count of 4.8×10^6 cells/mL. This was significantly lower than the 7.3×10^6 cells/mL recorded in rural bees exposed to much cleaner air (PM2.5: 28 µg/m³, PM10: 60 µg/m³). Hemocytes are essential components of the insect immune system and play a vital role in pathogen defense, wound healing, and detoxification processes. The lower hemocyte density observed in polluted-site bees suggests that airborne pollutants may suppress immune function and increase physiological stress. Similar observations have been reported by Zhao et al. (2022), who found that environmental stressors can alter immune responses in Apis cerana, reducing their ability to cope with diseases and other environmental challenges.

Foraging performance was also affected. Urban bees averaged only 58 foraging trips per hour compared to 89 trips per hour for rural bees. This happens because pollution destroys flower scents. Flowers give off scent chemicals called VOCs so bees can find them. But gases in dirty air break these scents down fast, so the smell doesn't travel far. This supports what Fuentes et al. found in 2016. Particulate matter deposited on the antennae and body surfaces can interfere with sensory perception used for navigation and flower detection. Less efficient foraging reduces nectar and pollen intake, which can impact brood development, food stores, and long-term colony strength. These findings support previous studies showing that polluted environments disrupt normal bee behavior and reduce pollination efficiency.

Mortality rates were nearly three times higher in colonies located in the polluted area (14.5%) compared to rural areas (5.2%). Increased mortality may result from the combined effects of physiological stress, impaired immunity, and reduced access to nutritional resources. Continuous exposure to airborne contaminants can increase energy expenditure associated with detoxification and stress responses, thereby reducing longevity. The statistical significance ($p < 0.05$) confirms these differences are not due to random variation. Taken together, lower hemocyte density, reduced foraging, and higher mortality indicate that urban air pollution acts as a sublethal stressor on A. cerana colonies in this region.

Overall, the findings highlight the ecological consequences of declining air quality on pollinator health. Since honey bees are major contributors to crop pollination and ecosystem functioning, continued exposure to urban pollutants may reduce pollination services and threaten agricultural productivity. Therefore, measures aimed at improving air quality, reducing vehicular emissions, and conserving pollinator-friendly habitats are essential for maintaining healthy honey bee populations in rapidly

urbanizing regions such as Baghpat District.

5. Conclusion

This investigation revealed that exposure to urban air pollution adversely affects the physiological condition, behavior, and survival of Apis cerana populations in Baghpat District. Honey bees inhabiting areas with elevated particulate matter concentrations exhibited lower hemocyte densities, indicating weakened immune capacity when compared with bees from less polluted rural environments. In addition, polluted-site colonies showed reduced foraging activity and increased mortality, suggesting that airborne contaminants can interfere with normal biological functions and colony performance.

The observed decline in foraging efficiency may limit the collection of nectar and pollen, thereby affecting colony nutrition and overall productivity. Simultaneously, reduced immune competence and elevated mortality rates indicate that chronic exposure to polluted environments places substantial stress on honey bee populations. These combined effects have the potential to diminish pollination services, which are essential for maintaining agricultural yields and ecosystem functioning.

The findings emphasize the need for greater attention to air quality management in urbanizing regions. Reducing particulate emissions, promoting environmentally sustainable transportation practices, and conserving flowering habitats can help mitigate the negative effects of pollution on pollinators. Future research should incorporate broader geographic coverage, seasonal monitoring, and additional physiological indicators to improve understanding of the long-term consequences of air pollution on honey bee health and colony sustainability.

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