Environmental Toxicology and Pharmacology 114 (2025) 104658

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Effects of anthropogenic stress on stingless bees *Melipona mandacaia* inhabiting urban and natural environments

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ARTICLE INFO

Edited by Dr. M.D. Coleman

Keywords:
Anthropogenic stressors
Biomarkers
Caatinga
Integrated Analysis
Pollinators

ABSTRACT

Bees play a crucial role as pollinators, significantly contributing to ecosystem health. However, they face growing threats from human activities. This study uses biomarkers to evaluate the health status of *Melipona mandacaia*, a stingless bee native to the Caatinga biome, as indicators of anthropogenic stress. Bees were collected from the unique Caatinga biome, which had no recorded human pressure, and from an urban area with high human pressure. These bees were then analyzed for various biomarkers to assess the different levels of anthropogenic stress. The biomarkers included cholinesterases (ChE) to assess neurotoxicity, catalase (CAT) to measure antioxidant responses, glutathione *S*-transferases (GST) for detoxification pathways, and lipid peroxidation (LPO) as an indicator of oxidative stress. The results reveal that ChE inhibition may be associated with stress levels due to human activities showing an inhibition pattern with increased stress levels (up to 54.4 % inhibition), while the remaining biomarkers showed mixed responses across the different stress-level areas. In addition, the use of a principal component analysis (PCA) allowed a separation between the different groups and the weigh of the measured variables to each anthropogenic stress group. The integrated biomarker response (IBR) index was applied showing a clear distinction among groups. The obtained results could be partly explained by the beekeeping practices in some locations, which may have mitigated the effects of anthropogenic stressors to a certain degree, especially in HS. These findings underscore the importance of monitoring wild bee health in the Caatinga and demonstrate the value of a multifaceted biomarker approach for understanding the impacts of anthropogenic stressors on bee populations in varied environments and the effects of beekeeping.

1. Introduction

Bees are essential pollinators for maintaining the health and functioning of ecosystems, the reproduction of native plants, agricultural production and food security (Potts et al., 2016; Murphy et al., 2022; Gekière et al., 2023). Their extensive foraging behaviour, which involves collecting nectar, pollen, resin, and water, also exposes them to diverse pollutants and environmental stressors (Badiou-Bénéteau et al., 2012; Carvalho et al., 2013; Gekière et al., 2023; La Porta et al., 2023). Consequently, bees serve as reliable bioindicators of environmental quality, detecting spatial and temporal variations in terrestrial ecosystems (Carvalho et al., 2013; La Porta et al., 2023).

Research on the effects of various stressors such as pesticides, heavy metals, or climate changes, primarily focuses on the species *Apis mellifera* (e.g., Gauthier et al., 2018, Tan et al., 2022; Benito-Murcia et al., 2024; Ayoub et al., 2024; Hisamoto et al., 2024; Mackei et al., 2024; Schuhmann and Scheiner, 2025). However, *A. mellifera* is native to Asia, Africa, the Middle East and Europe (Carr, 2023), and it does not accurately represent the diversity of more than 20,000 species (Gekière et al., 2023), like the Meliponine tribe. This tribe includes more than 600

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https://doi.org/10.1016/j.etap.2025.104658
Received 11 January 2025; Received in revised form 10 February 2025; Accepted 15 February 2025
Available online 17 February 2025

described species of eusocial bees (Ascher and Pickering, 2024; Roubik, 2023), with 40 species found in the Caatinga biome (Rodrigues et al., 2025) and 244 species found in Brazil (Ascher and Pickering, 2024).

Melipona mandacaia, a stingless bee endemic to the Caatinga, is an important representative of native Brazilian Meliponine species (Silveira et al., 2002; Grüter, 2020; Ascher and Pickering, 2024). This unique biome covers approximately 54 % of northeastern Brazil and is characterised by a high number of endemic flora and fauna adapted to the warm and dry weather of the region (Alves et al., 2006; Carneiro-neto et al., 2017; Melo et al., 2023). However, significant portions of its original habitat have been lost due to fragmentation and human disturbances, directly impacting native bee populations (Antongiovanni et al., 2020, 2022). *M. mandacaia* is an ecologically and economically important species, serving as a pollinator for numerous native and cultivated plant species within the Caatinga biome (Silveira et al., 2002; Grüter, 2020). Its role makes it a valuable bioindicator for assessing the environmental health of this biome (Silveira et al., 2002; Grüter, 2020; Ascher and Pickering, 2024).

Biomarkers are an effective tool for assessing the health status of bioindicators, as they provide insights into environmental health by reflecting the impacts of anthropogenic stressors across various biological levels observed in different bee species (Badiou-Bénéteau et al., 2012; Carvalho et al., 2013; Caliani et al., 2021b; Lupi et al., 2021; La Porta et al., 2023). Commonly used biomarkers include the activity assessment of enzymes, such as cholinesterase (ChE) for neurotoxicity analysis, catalase (CAT) for antioxidant response evaluation, glutathione *S*-transferases (GST) for detoxification, and lipid peroxidation (LPO) rates for oxidative stress evaluation (Badiou-Bénéteau et al., 2012; Lupi et al., 2021). Biomarkers have enabled the assessment of various environmental stressors on bee populations, such as urban and industry pollution (Nikolić et al., 2016; Al Naggar et al., 2020; Li et al., 2024) or pesticide exposure (Gauthier et al., 2018; Tan et al., 2022; Benito-Murcia et al., 2024; Ayoub et al., 2024; Hisamoto et al., 2024; Mackei et al., 2024). This evidence supports the use of bees as models for analysing biomarkers related to environmental contamination (Badiou-Bénéteau et al., 2012; Carvalho et al., 2013; La Porta et al., 2023). To enhance assessments of anthropogenic impacts on bee populations, an integrated approach that combines multiple biomarkers is essential for achieving a more accurate diagnosis of exposure to environmental stressors (Badiou-Bénéteau et al., 2012; Lupi et al., 2021).

Considering the ongoing degradation of the Caatinga biome in recent decades, coupled with significant anthropogenic pressures and the pivotal ecological role of *M. mandacaia* both within this ecosystem and for local communities, this study aims to evaluate the effects of different levels of anthropogenic stress on key biomarkers.

2. Methodology

2.1. Sampling

In March 2023, during the region's precipitation period, stingless bees of the species *Melipona mandacaia* were collected from two distinct locations, as detailed in Table 1. The degree of anthropogenic stress varied between these locations, depending on factors such as local human activities (e.g., farming, cattle breeding), air pollution, and other forms of human interaction. It was considered that higher levels of human interaction and proximity corresponded to greater anthropogenic impact.

2.2. Laboratory analyses

The sample processing protocol has been previously described in detail by Ferreira et al. (2010). Briefly, 30–50 replicates were measured for each stress group, with each replicate corresponding to a single bee (as detailed in Table 1). Bees were weighed and then sectioned, with the head homogenised for ChE analysis and the body used to analyse the remaining biomarkers. The samples were homogenised in a Tris-HCl buffer (pH 7.4, 50 mM), and centrifuged at 10,000 g (4°C) for 10 minutes. The supernatants were then stored at −80°C. At the time of analysis, the frozen samples were thawed and maintained at a cool temperature throughout all subsequent procedures. The lipid peroxidation (LPO) assay was adapted to a microplate format based on the methods described by Bird and Draper (1984) and Ohkawa et al. (1979). The activity of glutathione *S*-transferases (GST) was determined as described by Habig et al. (1974). Catalase (CAT) activity was measured based on the method described by Clairborne (1985), also adapted to a microplate format. The cholinesterase (ChE) activity was determined according to the Ellman method (Ellman et al., 1961). Protein concentration for all biomarkers was determined using the Bradford method (Bradford, 1976) using bovine serum albumin as the standard.

2.3. Statistical analyses

The biomarker data were evaluated for the presence of outliers, with only values within the quartile$_{(1\ \text{and}\ 3)}$ ± interquartile range being maintained. Biomarker's data was checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). When data did not show a normal distribution or homoscedasticity, the Kruskal-Wallis test was compared between the groups. In the event of statistical significance ($p < 0.05$), the medians of the groups were compared using Dunn's post-hoc test. Afterwards, the variable matrices were standardised (z-score) and analysed using principal component analysis (PCA). Missing values are imputed by the mean of the variable. In the PCA, factor loadings were defined, representing the linear correlations of each variable with the composition of the factor. Here, the factor is a new latent variable defined by the set of factor loadings. The factor loadings resulting from the first two principal components were assessed for significance using Single Factor Analysis of Variance (ANOVA), given that these variables met the assumptions of normality and homoscedasticity. In the event of statistical significance, the Tukey's Non-Honest Significant Difference (Tukey-NHSD) post-hoc test was carried out. All statistical tests were performed at an $\alpha = 0.05$ using the R software (R Core Team, 2022). Based on the biomarker variables, the Integrated Biomarker Response Index - IBR was applied according to Beliaeff and Burgeot (2002).

3. Results

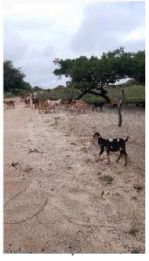
The average weight of bees decreased across the different treatment groups, as follows: NS (54.4 ± 5.78 mg) > HS (52.7 ± 5.31 mg) > MS (51.2 ± 6.11 mg) > LS (49.4 ± 4.31 mg). Statistical analysis revealed significant differences among the groups ($\chi^2 = 37.2618$, df = 3, $p < 0.001$). Particularly, the NS group was significantly different from all other groups, the LS group was also significantly different from all other groups, while the MS and HS groups were statistically identical.

The analysis of cholinesterase activity (Fig. 1A) showed significant differences among groups ($\chi^2 = 45.06$, df = 3, $p < 0.0001$). Bees from the Caatinga's meliponary (LS) exhibited similar cholinesterase (ChE) activity levels to bees from the urban meliponary (HS) but significantly differed from the MS and NS areas. The highest ChE activity occurred in bees collected from the no-observed stress area (NS), while the lowest activity was observed in the moderate-stress area (MS). Catalase activity (Fig. 1B) showed significant differences among groups ($\chi^2 = 17.81$, df = 3, $p < 0.0001$). The LS area showed lower enzyme activity than the other groups, which were statistically similar. The GST activity showed a significant difference among groups ($\chi^2 = 67.75$, df = 3, $p < 0.0001$). The activity was higher in the HS area with strong anthropogenic stress than in the other statistically identical areas (Fig. 1C). As for LPO rates, the analysis revealed significant differences among groups ($\chi^2 = 10.64$, df = 3, $p = 0.01$ – Fig. 1D). The LS area showed LPO rates statistically different from the other areas. Meanwhile, the NS, MS, and HS areas all exhibited comparable LPO rates.

The principal component analysis of the biomarkers revealed that

Table 1
Environmental stress gradient as a result of anthropogenic activity to which *Melipona mandacaia* were exposed.

ENVIRONMENTAL STRESS LEVEL

Stress Levels:	Area Description;
No observed anthropogenic stress (NS) 	**Location:** Casa Nova (BA) **Centroid geographical coordinates:** -9.400428, -41.386407 **Average temperature (1 month prior to sampling):** 28,9°C **Cumulative rainfall (1 month prior to sampling):** 16,9 mm **Description:** Natural Caatinga area, far from any anthropogenic disturbances (e.g., residences, plantations, cattle breeding). Bees were collected from hives built within *Commiphora leptophloeos* (umburana) trees. **Number of hives: 3** **Number of organisms per hive: 10** **Total number of sampled organisms: 30**
Low anthropogenic stress (LS) 	**Location:** Casa Nova (BA) **Centroid geographical coordinates:** -9.400428, -41.386407 **Average temperature (1 month prior to sampling):** 28,9°C **Cumulative rainfall (1 month prior to sampling):** 16,9 mm **Description:** Borderline between a farm and the Caatinga biome, with some exposure to anthropogenic activities, as a result of the nearby activities (e.g., pesticide application, cattle breeding). Bees were collected from meliponiculture boxes that were occasionally visit by the farmer to collect its products. **Number of hives: 4** **Number of organisms per hive: ~12** **Total number of sampled organisms: 50**
Moderate anthropogenic Stress (MS) 	**Location:** Casa Nova (BA) **Centroid geographical coordinates:** -9.400428, -41.386407 **Average temperature (1 month prior to sampling):** 28,9°C **Cumulative rainfall (1 month prior to sampling):** 16,9 mm **Description:** Rural area, in the middle of a farm with agricultural practices and cattle breeding (goats). Bees were collected from meliponiculture boxes that were regularly visited by the farmer to collect its products, but no artificial food (e.g., water with sugar) was offered. **Number of hives: 3** **Number of organisms per hive: 10** **Total number of sampled organisms: 30**
High anthropogenic Stress (HS) 	**Location:** Petrolina (PE) **Centroid geographical coordinates:** -9.3713750, -40.4839200 **Average temperature (1 month prior to sampling):** 29,5°C **Cumulative rainfall (1 month prior to sampling): 4.4 mm** **Description:** Urban area of Petrolina, located within a city area with different levels of anthropological impact, such as car traffic, wastewater treatment stations and even a fragment of the native Caatinga biome. Bees were collected from meliponiculture boxes that were regularly visited by the farmer to collect its products, with artificial food (water with *Apis mellifera* honey in a 1:1 ratio) being offered whenever necessary. **Number of hives: 5** **Number of organisms per hive: 10** **Total number of sampled organisms: 50**

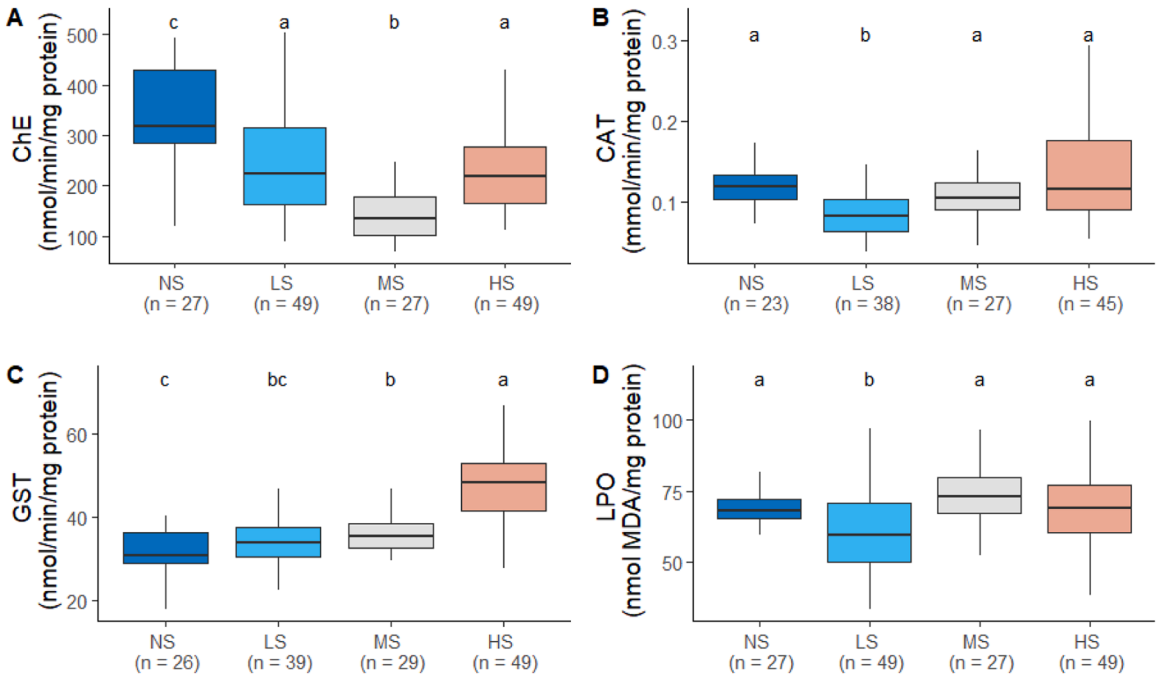


Fig. 1. Boxplot comparing the biomarker's activity/rates in *Melipona mandacaia* from different study areas. ChE – denotes cholinesterases; CAT – denotes catalase, GST – denotes glutathione *S*-transferases, LPO – denotes lipid peroxidation, NS - denotes no-observed stress area (dark blue), LS - denotes low-stress area (light blue), MS - denotes moderate-stress area (orange), HS - denotes high-stress area (red). Different letters denote different groups ($p < 0.05$).

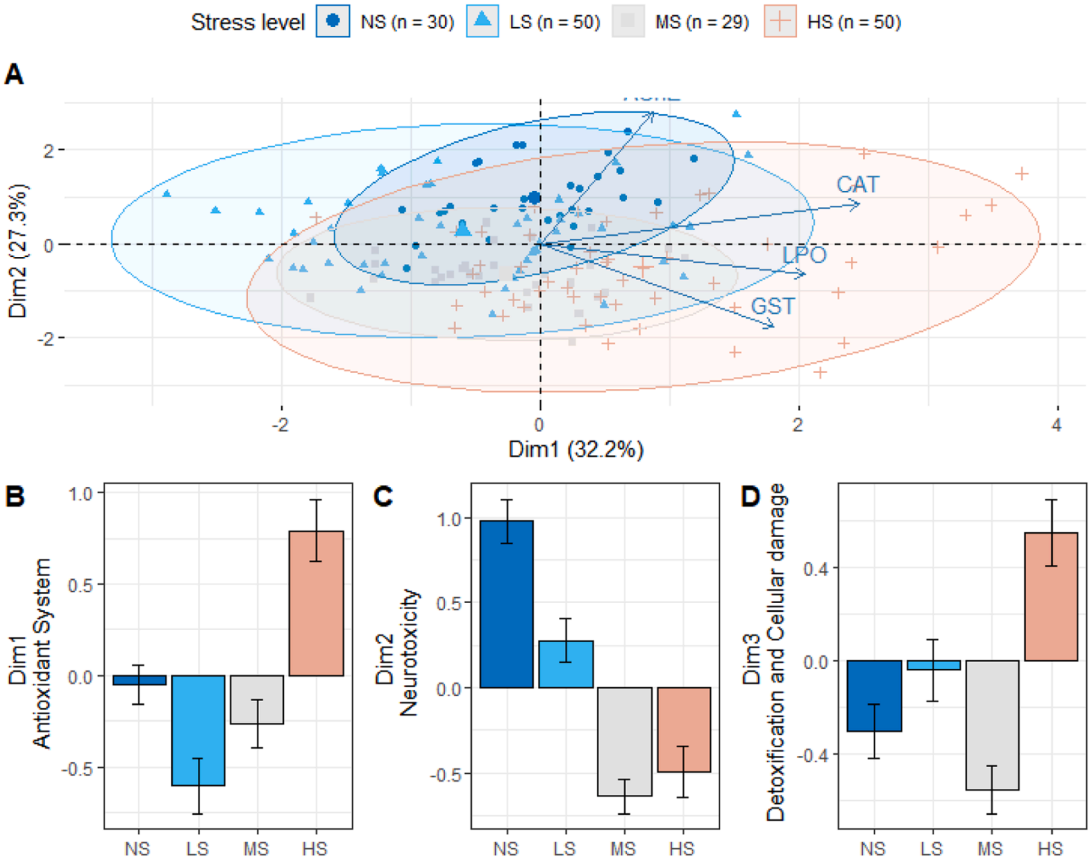


Fig. 2. A) Principal Component Analysis (PCA) and projections of the main dimensions based on the activity of biomarkers in *Melipona mandacaia* from different study areas. Mean projections and standard deviations of the areas studied in B) Dimension 1 – Antioxidant System; C) Dimension 2 – Neurotransmission; and D) Dimension 3 – Detoxification and Cellular damage.

the first principal component (Dimension 1) was primarily driven by the positively correlated CAT variable, which can be interpreted as representing "Antioxidant System" (Eigenvalue = 1.29; Variance = 32.19 %). The second principal component (Dimension 2) showed a greater contribution from the ChE variable, with positive scores indicating higher enzymatic activity. This dimension was labelled "Neurotransmission" (Eigenvalue = 1.09; Variance = 27.32 %). The third principal component (Dimension 3) showed a greater contribution from the GST and LPO variables, with negative scores indicating higher stress. This dimension was labelled "Detoxification and Cellular damage" (Eigenvalue = 0.88; Variance = 21.95 %; Fig. 2A).

The analysis of the latent variables "Dim.1 – Antioxidant System", "Dim. 2 – Neurotransmission" and "Dim. 3 – Detoxification and Cellular damage" revealed statistically significant differences among the study areas ($F_{3,155} = 17.50$, $p < 0.0001$; $F_{3,155} = 24.85$, $p < 0.0001$; $F_{3,155} = 12.31$, $p < 0.0001$, respectively). Dim 1 (Antioxidant System) was primarily influenced by CAT, with the HS group showing the highest factor loadings compared to the other groups ($p < 0.05$). Dim 2 (Neurotransmission) was predominantly influenced by ChE, with the NS and LS groups displaying higher factor loadings, indicating greater ChE activity and being statistically distinct from the MS and HS groups ($p < 0.05$). Dim 3 (Detoxification and Cellular damage) was mainly influenced by GST and LPO, with the HS group showing the highest factor loadings compared to the other groups ($p < 0.05$).

As shown in Fig. 3, the Integrated Biomarkers Responses (IBR) index varied across the four stress areas (NS, LS, MS, and HS). The MS area exhibited the highest IBR value (5.06), followed by HS (2.80), LS (1.36), and NS (0.00).

4. Discussion

The potential impacts of human-driven activities on wildlife have been a central focus within environmental toxicology (Refati et al., 2023). While human pressures do not always result in negative outcomes, they may instead lead to adaptations and the establishment of new homeostatic states among affected organisms (Hammond et al., 2020). To investigate the impacts of anthropogenic activity on native bee populations, this study evaluated four areas within the unique Caatinga biome that exhibited varying levels of environmental stress and human-induced pressures, assessing key biomarkers as indicators. The gradient of stress (NS < LS < MS < HS) was not entirely validated by the assessed biomarkers, PCA and IBR analyses as expected, but still distinguishing environmental conditions and bees' physiological responses. Although biomarkers can effectively reflect the state of the environment, isolating and determining the causes of the physiological conditions is challenging (Badiou-Bénéteau et al., 2012; Lupi et al., 2021; La Porta et al., 2023), especially in situations where organisms are under the care of keepers who aim to promote their welfare.

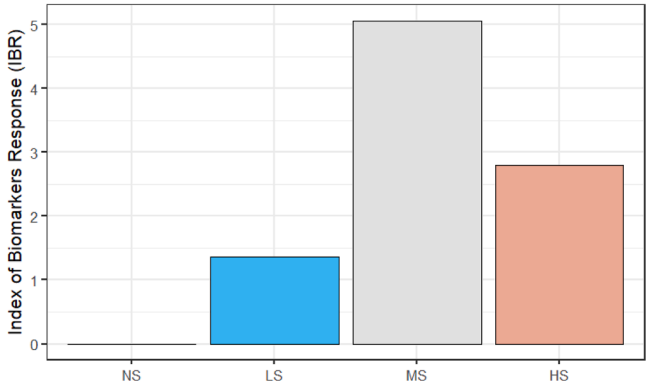


Fig. 3. Integrated Biomarkers Response (IBR) index for *Melipona mandacaia* under different stress areas. NS – no-observed-stress area (dark blue), LS – low-stress area (light blue), MS - moderate-stress area (orange), HS – high-stress area (red), ChE: cholinesterases, GST: glutathione *S*-transferases, LPO: lipid peroxidation, CAT: catalase.

Cholinesterases (ChE) are widely used biomarkers of neurotransmission. They were employed in this study as numerous stressors can inhibit their activity, including insecticides (Carvalho et al., 2013; Zhu et al., 2017; Lv et al., 2023; Ayoub et al., 2024; Benito-Murcia, 2024), fungicides (Caliani et al., 2021a; Lv et al., 2023; Martins et al., 2023), and other xenobiotics from urbanisation and industrial processes, such as cadmium and lead (Al Naggar et al., 2020; Li et al., 2024). Previous studies have reported similar inhibition of ChE in other species, as report below. For example, Caliani et al. (2021b) evaluated honeybee health under different anthropogenic pressures (a wood – reference site, an orchard, an agricultural, and an urban area), finding more pronounced ChE inhibition in agricultural areas than urban areas (both lower than the woodland), likely due to higher pesticide levels in agricultural areas versus the higher levels of hydrocarbons and metals emitted from fuel combustion of cars or wood burning in urban areas. The LS area exhibits considerable variation. While a single cause cannot be identified, this variation may stem from environmental factors (e.g., temperature, sunlight, humidity) or differing levels of human impact across the individual colonies (Belsky and Joshi, 2019; Meikle et al., 2020; Alves et al., 2023). The inhibition of cholinesterase (ChE) has significant neurological repercussions, including impaired motor function, reduced foraging, cognitive deficits, altered behaviours, and overall diminished fitness in affected organisms (Gashout et al., 2020; Caliani et al., 2021b; Tan et al., 2022; Ayoub et al., 2024).

As for the detoxification enzymes GST, a slight induction pattern was observed with increased stress levels, although only significant for the HS area. Previous studies that exposed bees in the laboratory to chemical compounds (Cd and Pb) associated with urbanisation had reported an induction in GST activity levels (Li et al., 2024). Similarly, field studies have also shown the induction of GST activities in bees exposed to several pesticides (e.g., Caliani et al., 2021; Lupi et al., 2021). This enzyme plays a key role in the phase two catalysis of numerous xenobiotics (Caliani et al., 2021a; Benito-Murcia, 2024). This process results in more polar compounds' excretion, thereby helping protect cells against oxidative damage (Badiou-Bénéteau et al., 2012; Han et al., 2019; Benito-Murcia, 2024). As with the previous biomarker, there is no single identifiable cause for the similar levels of GST activity observed no-obsersed stress, low-stress and moderate-stress levels. This is especially true when pesticides and combustion-derived metals are a major driver of GST induction. Nonetheless, the involvement of other antioxidant enzymes like glutathione peroxidase (GPx), glutathione reductase (GR), and catalase, as well as altered glutathione (reduced and oxidised) levels, are important factors to consider when analysing GST responses (Ferreira et al., 2015a; Ferreira et al., 2015b; Nikolić et al., 2016; Lupi et al., 2021).

The antioxidant enzyme catalase (CAT) plays a crucial role in the antioxidant system by converting hydrogen peroxide (H_2O_2) into harmless substances, thereby eliminating reactive oxygen species (Su et al., 2019). However, exposure to environmental contaminants can alter CAT function (Carvalho et al., 2013; Su et al., 2019). In the present study, CAT activities were significantly lower in the low-stress (LS) area, followed by an increasing pattern as stress levels increased. This inhibition may result from the high quantity of superoxide radicals (O2•-) already present (Geret et al., 2002; Grilo et al., 2020). A U-shaped activity curve was also observed, where increased stress levels initially inhibited and then induced CAT activity. Similar trends in CAT activity have been reported in other studies. Lupi et al. (2021) showed significantly elevated CAT levels in chemically stressed bee pupae compared to those with no stress or multi-stress conditions. Conversely, El-Saad et al. (2017) detected the lowest CAT levels in heavily urbanised sites with prolonged pesticide exposure, suggesting anthropogenic impacts can reduce this antioxidant enzyme. Moreover, species-specific adaptive CAT responses to environmental stressors have been documented. For

example, the bee species *Apis dorsata* exhibited significantly increased CAT in high-intensity cropping areas, while *Apis cerana* showed the opposite, an inhibition of CAT activity (Chakrabarti et al., 2015). These findings indicate that bee antioxidant defences, as reflected by CAT activity, are highly context-dependent and influenced by local ecological dynamics and stress exposure levels.

Despite the efforts of GST and CAT to detoxify and handle oxidative stress, anthropogenic activity may still induce elevated lipid peroxidation (LPO) rates, negatively affecting organismal health (Morgado et al., 2013). Except for the LS area, which had a lower mean value but high variation, all other areas exhibited similar LPO rates. In this study, the "protective" effects of GST and CAT were mainly observed within the HS area, leading to similar LPO rates as those seen in almost all the other areas. As expected, the previous enzyme activity results would indicate that increased stress levels, whether from oxidative stress due to human handling, pesticide exposure, or other xenobiotics from fuel combustion and wood burning, would result in increased LPO rates. For example, *Apis mellifera* and *Tetragonisca angustula* showed clear signs of oxidative stress when exposed to similar doses of fipronil, with a more marked increase in LPO rates observed in *T. angustula* (Mena et al., 2023).

When multiple biomarkers are used or subtle differences are observed, an integrative analysis like Principal Component Analysis (PCA) can help explain and visualise patterns and interactions among variables. This multivariate statistical approach is useful for natural studies involving dynamic, heterogeneous environments, as it allows simultaneous assessment of multiple factors, going beyond univariate analyses. Integrating biomarker data helps understand how bees respond to environmental pressures, identify areas of major ecological vulnerability, and prioritise management and conservation interventions. PCA analysis contributes to a more comprehensive understanding of environmental impacts, supporting the development of effective conservation strategies. The PCA revealed three key variation patterns in the *M. mandacaia* biomarkers: Dimension 1 related to antioxidant responses (CAT), Dimension 2 associated with neurotransmission (ChE), and Dimension 3 linked to detoxification and cellular damage (GST and LPO). These results suggest that bees in areas with greater human impact (HS) exhibited elevated antioxidant and detoxification responses and increased cellular damage, likely due to an insufficient capacity of these systems. Additionally, the neurotransmission system in these bees showed a negative response. In contrast, bees from the less impacted (NS) area demonstrated a robust neurotransmission response. The PCA analysis findings were confirmed by the IBR index. Here, the NS area had a zero score, which increased slightly in the LS area and nearly quadrupled in the MS area before dropping to about half that in the HS area. The lower HS area index may stem from the beekeeper's positive influence on the colonies rather than the actual impact of the previously mentioned stressors within the different stress zones. Still, it is important to highlight that these animals live in an urban meliponiculture and are directly fed glucose syrup. The HS group has higher weight and protein levels than the other groups, but this does not necessarily mean they are healthier.

Overall, the results obtained from this study show that the positive impact of beekeeping may counteract the increase in stress levels. With the clear exception of the ChE, biomarkers responded in an even manner, with slight inductions or inhibitions and very similar rates of cellular damage. The integrated analysis corroborated these findings, grouping PCA dimensions by biological traits, and IBR showed that beekeeping activity can reduce stress levels. Other collected variables, such as the average weight of bees, support these results. Here, the NS group presents the highest average weight, which is then followed by a decrease in beekeeping activity. The HS colonies had the most active beekeeping practices, regularly feeding bees sugar solutions and commercial bee food as needed. The HS colonies showed similar weights to MS colonies, which had moderate beekeeping activity, with farmers providing the same supplemental food but not so frequently. In contrast, the LS colonies had the lowest level of beekeeping activity, with only occasional contact with beekeepers, as described in the methodology. The results of this study, conducted during the wet season when bees had greater access to floral resources, may differ from findings that could emerge in the dry season. In fact, the expected results may trend in the opposite direction of stress levels, with healthier colonies with high beekeeping activity exhibiting better status compared to those in the Caatinga biome (NS).

While the observed data offers valuable insights, this study has limitations that should be further investigated. Although the different anthropogenic stress levels could be clearly delineated, the assessment of specific contaminants was hindered by quantification limits and the decision to avoid destroying any bee colonies to collect honey and other products.

5. Conclusion

This study is a crucial first step in developing new research that correlates biomarkers with ecological variables across habitats and seasons. The work aims to provide key information on how sub-individual changes relate to higher organisational levels, such as behaviour, lifespan, and impacts on ecosystem services like pollination rates, honey/pollen quality, and production. Understanding species-specific responses to environmental stressors will be vital for designing targeted conservation measures to protect not just *M. mandacaia* but also other native bee species that are essential to the health and resilience of the caatinga ecosystem and the ecosystem services in the San Francisco Valley region.

In conclusion, this study showed that *M. mandacaia* can be used as a bioindicator species to evaluate environmental quality in the Caatinga biome. Integrating biomarker analysis provides valuable information on the physiological impacts of anthropogenic stressors on bee populations, which individually only showed a clear impact on ChE activity, which can be important early warning signs to avoid major effects at the ecological level. Even as urbanisation and agricultural practices continue to expand, understanding these dynamics is essential for developing effective conservation strategies to protect bee health and biodiversity. By prioritising the conservation of key pollinators such as *M. mandacaia*, we can better protect the ecological health of vital ecosystems and ensure sustainable agricultural practices that benefit both humans and wildlife.

Ethics approval

This study used invertebrate species that do not require previous ethical approval. The animal experiments comply with the EU Directive 2010/63 for the protection of animals used for scientific purposes.

CRediT authorship contribution statement

Bittencourt Guimarães Ana Tereza: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **de Souza Isabelle Letícia Bender:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Macarini Leanna Camila:** Investigation, Formal analysis, Conceptualization. **de Oliveira Cíntia Mara Ribas:** Methodology, Conceptualization. **Ferreira Nuno G. C.:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the kind permission and assistance provided by the "*Associação de Apicultores e Meliponicultores da Fazenda Santarém*" and the "*Meliponário Sanctorum*" to conduct sampling on their lands and within their meliponaries. Their contributions, including reducing bee stress during capture and sharing valuable knowledge about the local area and bees' lifestyle, significantly enhanced this study's data collection and analysis.

Nuno G. C. Ferreira was supported by FCT through 2021.02111.CEECIND (DOI: 10.54499/2021.02111.CEECIND/CP1665/CT0003). Ana T. B. Guimarães was supported by CNPQ productivity researcher scholarship n. 304169/2020.

This work was supported by Fundação para a Ciência e Tecnologia (FCT) within the scope of UIDB/04423/2020, UIDP/04423/2020, Project BEESNESS (DOI:10.54499/CIRCNA/BRB/0293/201).

Data availability

Data will be made available at Zenodo - http://doi.org/10.5281/zenodo.14605065.

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