Effects of anthropogenic stress on stingless bees Melipona mandacaia (Hymenoptera: Apidae) inhabiting urban and natural environments - Dataset

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ARTICLE INFORMATION

Article title

Effects of anthropogenic stress on stingless bees *Melipona mandacaia* (Hymenoptera: Apidae) inhabiting urban and natural environments - Dataset

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Abstract

This dataset provides a comprehensive analysis of the impacts of human-induced stress on bee species native to the Caatinga biome. Bees play a vital role as pollinators, significantly contributing to ecosystem health, but they face growing threats from human activities. This study evaluates the health status of *Melipona mandacaia*, a stingless bee, using biomarkers as indicators of anthropogenic stress, comparing bees from a pristine Caatinga biome with no recorded human pressure, and an urban area with high human pressure. The biomarkers analysed included cholinesterases (ChE) for neurotoxicity, catalase (CAT) for antioxidant responses, glutathione *S*-transferases (GST) for detoxification pathways, and lipid peroxidation (LPO) as an indicator of oxidative stress. Additionally, the Integrated Biomarker Response (IBR v.1) index is used to provide a holistic assessment of bee health by integrating multiple biomarkers into a single measure.

Keywords

Anthropogenic stressors; Biomarkers; Caatinga;Integrated Analysis; Pollinators

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SPECIFICATIONS TABLE

Subject	Environmental Sciences and Biological Sciences: Management, Nature and Landscape Conservation, Pollution
Specific subject area	Environmental Toxicology
Type of data	Tables (.xlsx format) Raw and simplified variables created by consolidating raw data.
Data collection	The data set contains one file. This file contains the weight of the individuals and the data for the biomarkers ChE, CAT, GST and LPO, as well as the mean and standard deviation per group and an indication of outliers. These parameters were used to calculate the Integrated Biomarker Response Index (IBR).
Data source location	The samples for this study were collected from two locations in northeastern Brazil: Casa Nova, Bahia (Centroid geographical coordinates: -9.400428, -41.386407), and Petrolina, Pernambuco (Centroid geographical coordinates: -9.3713750, -40.4839200). The biomarkers data were acquired at the "Laboratório de Investigações Biológicas" (Laboratory of Biological Investigations) at the Universidade Estadual do Oeste do Paraná, located in Cascavel, Parana, Brazil.
Data accessibility	Data is available within this manuscript and also at Repository name: ZENODO Data identification number: 10.5281/zenodo.18404353 Direct URL to data: https://doi.org/10.5281/zenodo.18404353
Related research article	I.L. Bender de Souza, L.C. Macarini, C.M.R. de Oliveira, N.G.C. Ferreira, A.T.B. Guimarães, Effects of anthropogenic stress on stingless bees *Melipona mandacaia* inhabiting urban and natural environments, Environmental Toxicology and Pharmacology 114 (2025) 104658. https://doi.org/10.1016/j.etap.2025.104658.

VALUE OF THE DATA

- Provides crucial baseline information on *M. mandacaia* health status in relation to environmental stressors in the Caatinga biome.
- This data provides valuable insights into how varying degrees of human activity impact the biological indicators of *M. mandacaia*, a stingless bee species native to the Caatinga biome.
- Offers insights into a native stingless bee species, expanding knowledge beyond the commonly studied *Apis mellifera*.

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- This research aligns with and supports other studies on various bee species that examine the effects of different levels of anthropogenic activity on bee health and biology.
- This data provides valuable insights for ecological risk assessors and policymakers, enabling them to better understand and consider environmental stressors' subtle, non-lethal effects on *M. mandacaia* and other species native to the Caatinga biome.

BACKGROUND

Bees constitute essential components of healthy ecosystems, fulfilling critical functions in plant reproduction, agricultural productivity, and food security [1-3]. Their extensive foraging behaviour exposes them to diverse pollutants [3-6], positioning them as valuable bioindicators of environmental quality [5-6]. While the majority of research prioritises the honeybee (*Apis mellifera*), a species that encompasses only a fraction of bee diversity. It particularly overlooks the stingless bees of the Meliponini tribe. *Melipona mandacaia*, a stingless bee endemic to Brazil's Caatinga biome, is a significant ecological and economic resource due to its pollination of both native and cultivated plant species. Nevertheless, the Caatinga biome faces substantial threats from habitat degradation and anthropogenic disturbances, thereby jeopardising these crucial bee populations [7-9].

To better understand these impacts, the present study investigates how varying intensities of anthropogenic stress modulate key physiological biomarkers in *M. mandacaia*. Enzymatic biomarkers, encompassing neurotoxicity indicators, antioxidant responses, detoxification pathways, and oxidative stress markers, facilitate assessment of environmental health status and the physiological consequences of stressor exposure in bee organisms. Evaluation of these biomarkers enables the identification of pollution and pesticide-induced effects at the physiological level. Through an integrated biomarker approach that synthesises multiple stress indicators, researchers can achieve a comprehensive understanding of how environmental stressors influence bee population dynamics, particularly given the accelerating degradation of the Caatinga and the ecological significance of *M. mandacaia*.

DATA DESCRIPTION

The presented dataset includes two files as follows:

File: Biomarkers_Data_Final.xlsx – contains the raw and processed biomarker's data as follows:

- **Manuscript -** publication information.
- **Biomarkers_data -** Biomarker raw data for cholinesterases (ChE), catalase (CAT), glutathione S-transferases (GST) and lipid peroxidation (LPO) per individual.
- **Weight_data -** Weight data per individual.
- **Legend -** Description of the acronyms used in the tables.

File: Dataset_Zenodo.pdf – provides complete dataset documentation with general information.

EXPERIMENTAL DESIGN, MATERIALS AND METHODS

The full methodology can be read from the original manuscript Souza et al. (2025). Herein in a brief description:

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Sampling

Field sampling occurred during March 2023, coinciding with the regional wet season in northeastern Brazil. *Melipona mandacaia* specimens were obtained from two geographically distinct locations as detailed in Table 1. These areas were characterised by varying levels of anthropogenic stress based on local human activities (e.g., agriculture, cattle breeding, urbanisation), air pollution levels, and the intensity of human interaction.

Table 1- Characteristics of sampling areas from Souza et al. (2025)[10].

	No observed anthropogenic stress (NS)	**Low anthropogenic stress (LS)**	**Moderate anthropogenic Stress (MS)**	**High anthropogenic Stress (HS)**
Location	Casa Nova (BA)	Casa Nova (BA)	Casa Nova (BA)	Petrolina (PE)
Coordinates	-9.400428, -41.386407	-9.400428, -41.386407	-9.400428, -41.386407	-9.3713750, -40.4839200
Environmental Type	Natural Caatinga biome	Borderline (Farm / Caatinga)	Rural Area (Farm)	Urban Area (City)
Dominant Stressor	None (Pristine)	Nearby pesticide application, cattle breeding	Agricultural practices, cattle breeding (goats)	Car traffic, wastewater stations, high human density
Nesting	Natural hives in *Commiphora leptophloeos* (umburana) trees	Meliponiculture boxes	Meliponiculture boxes	Meliponiculture boxes
Human interaction	None (Wild)	Occasional visits	Regular visits	Daily visits
Dietary supplentation	None (Natural foraging)	None (Natural foraging)	No artificial food offered	Artificial food offered (water + Apis mellifera honey 1:1)
Average temperature (1 month prior to sampling)	28.9 °C	28.9 °C	28.9 °C	29.5 °C
Cumulative rainfall (1 month prior to sampling)	16.9 mm	16.9 mm	16.9 mm	4.43 mm
Sample size (hives)	3	4	3	5
Sample size (bees)	10 per hive (Total: 30)	~12 per hive (Total: 50)	10 per hive (Total: 30)	10 per hive (Total: 50)

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Laboratory analyses

Upon collection, samples were processed following the protocol described by Ferreira et al. (2015) [11]. For each stress group, individual bees were weighed prior to analysis. The head of each organism was separated for cholinesterase (ChE) quantification, while the body was homogenised for the analysis of the remaining biomarkers.

Lipid Peroxidation (LPO) was assessed using a microplate adaptation of the methods described by Bird and Draper (1984) [12] and Ohkawa et al. (1979) [13] to quantify TBARS levels. The enzymatic activity of Glutathione *S*-transferases (GST) was determined following the method of Habig et al. (1974) [14]. Catalase (CAT) activity was measured using a microplate-adapted method based on Clairborne (1985) [15]. Neurotoxicity was assessed by determining Cholinesterase (ChE) activity according to the method of Ellman et al. (1961) [16]. Total protein concentration for all enzymatic assays was determined using the Bradford (1976) [17] method, using bovine serum albumin (BSA) as the standard.

Post mitochondrial supernatant (PMS)

Each replicate consisted of two organisms, which were homogenised with a sonicator in 1 mL of 0.1 M K phosphate buffer (pH 7.4). From each homogenate, 150 µL were transferred to a microtube and supplemented with 5 µL of 4% (w/v) butylated hydroxytoluene (BHT) in methanol for the determination of lipid peroxidation (LPO). The remaining homogenate (850 µL) was centrifuged at 10,000 g for 20 min at 4 °C to obtain the PMS. The PMS was aliquoted into two microtubes for subsequent biomarker assays and protein quantification. All aliquots were stored at -80 °C for up to 2 weeks prior to analysis.

Lipid peroxidation (LPO)

The reaction mixture contained 150 µL of tissue homogenate, 500 µL of 12% (w/v) trichloroacetic acid (TCA) sodium salt, 500 µL of 0.73% (w/v) 2-thiobarbituric acid (TBA) and 400 µL of 60 mM Tris-HCl containing 0.1 mM diethylenetriaminepentaacetic acid (DTPA). Reactions were incubated at 100 °C in a water bath for 1 h, then centrifuged at 11,500 rpm for 5 min at 25°C. Samples were protected from light, maintained at 25 °C and read immediately at 535 nm. LPO was expressed as nmol TBARS hydrolysed per minute per mg wet weight, using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Glutathione *S*-transferases (GST)

Post mitochondrial supernatant (100 µL) was added to 200 µL of reaction medium, and the product formation was followed at 340 nm. The reaction medium consisted of 4.95 mL of 0.1 M K-phosphate buffer (pH 6.5), 900 µL of 10 mM reduced glutathione (GSH), and 150 µL of 10 mM 1-chloro-2,4-dinitrobenzene (CDNB). Enzymatic activity was expressed as units (U) per mg protein, where one unit corresponds to 1 nmol of substrate conjugated per minute, assuming a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Catalase

Post mitochondrial supernatant (50 µL) was added to a cuvette containing 500 µL of 0.03 M H_2O_2 and 950 µL of 0.05 M K-phosphate buffer (pH 7.0). The decomposition of H_2O_2 was followed at 240 nm. Catalase activity was expressed as U per mg protein, where one unit is defined as the decomposition of 1 µmol of substrate per minute, with a molar extinction coefficient of 40 M^{-1} cm^{-1}.

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Cholinesterases (ChE)

For each sample, one bee head was homogenised with a sonicator in 500 µL of 0.1 M potassium phosphate buffer (pH 7.2). Supernatants obtained after centrifugation at 3800 g for 3 min at 4 °C were collected and stored at -80 °C until analysis. In a 96-well microplate, 250 µL of reaction solution was added to 50 µL of sample and absorbance at 414 nm was recorded after 10, 15 and 20 min. The reaction solution contained 1 mL of 10 mM 5,5'-dithiobis (2-nitrobenzoic acid) - DTNB, 1.280 mL of 0.075 M acetylthiocholine iodide, and 28.920 mL of 0.1 M phosphate buffer. ChE activity was expressed as U per mg protein, where one unit corresponds to 1 nmol of substrate hydrolysed per minute, using a molar extinction coefficient of 1.36×10^3 M^{-1} cm^{-1}.

Protein quantification for biomarkers

For all biomarker assays, protein concentration was quantified using the Bradford method (Bradford, 1976), following the Bio-Rad Bradford micro assay protocol adapted to 96-well flat-bottom plates, with bovine γ-globulin as the protein standard.

Enzymatic activity calculations

Enzymatic biomarker activities were determined using spectrophotometric methods and calculated following the Beer-Lambert Law. The general approach for all biomarker assays involved measuring the change in absorbance over time, which was then converted to enzymatic activity expressed in units per mg of protein (U mg^{-1}). The calculation of enzymatic activity follows the equation:

$$Activity\ (U) = \frac{\Delta Abs/\Delta time}{\varepsilon \times l} \times \frac{V}{C_{pr} \times DF_{sample}}$$

where $\Delta Abs/\Delta time$ is the change in optical density per minute (the linear portion of the reaction); ε is the molar extinction coefficient (M^{-1} cm^{-1}) specific to each biomarker assay; l is the path length of the microplate well (0.9 cm); V is the reaction volume (mL); C_{pr} is the protein concentration (mg mL^{-1}) determined via the Bradford assay, and DF_{sample} is the dilution factor applied to the sample before analysis. The final activity is expressed as nmol of substrate converted per minute per mg of protein (U mg^{-1}), where one unit corresponds to the enzymatic conversion of 1 nmol of substrate per minute under the specified assay conditions. For each biomarker, the specific molar extinction coefficient and reaction wavelength employed are detailed in the respective sections above. The linear portion of the reaction kinetics was always selected for calculations to ensure that enzyme activity remained in the initial velocity phase, providing a reliable estimate of specific enzymatic performance independent of total protein concentration in the sample.

Integrated Biomarkers response index (IBR)

To provide an integrated assessment of environmental stress, the Integrated Biomarker Response (IBR) index (IBR) was calculated using the biomarker variables, following the methodology of Beliaeff and Burgeot (2002) [18]. All calculations were performed using Microsoft Excel®.

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LIMITATIONS

The dataset focuses on a single stingless bee species from a specific geographic region and time period, and represents responses to anthropogenic stressors in that particular context. Users should be aware that:

1) The findings may not be directly transferable to other *Melipona* species or geographic regions.
2) Enzymatic activities reflect acute exposure conditions at the time of collection, similar to a photographic record, and may not capture chronic or cumulative stress responses.
3) The biomarkers measured are indicative of physiological stress but do not constitute a complete assessment of individual or colony health.

Despite these limitations, the detailed raw data presented here offer significant value to the scientific community by enabling reproducible research, fostering collaborative meta-analytical efforts and advancing our understanding of how stingless bees respond to anthropogenic environmental change.

ETHICS STATEMENT

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 AUTHOR STATEMENT

I.L.B.S.: Validation, formal analysis, investigation, writing - original draft, visualisation; **L.C.M.:** Investigation and formal analysis; **C.M.R.O.:** Conceptualisation, methodology; **N.G.C.F.:** Conceptualisation, methodology, validation, formal analysis, resources, data curation, writing - original draft, supervision, project administration, funding acquisition; **A.T.B.G.:** Conceptualisation, methodology, validation, formal analysis, resources, data curation, writing - original draft, visualisation, supervision, project administration, funding acquisition. All authors contributed to the review and editing of the original manuscript.

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DECLARATION OF COMPETING INTERESTS

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.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this study, the author(s) used Gemini and Perplexity to improve the language and clarity of the manuscript. The author(s) reviewed and edited all content as needed and take(s) full responsibility for the content of the published article.

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