



# Mercury in honey of stingless bee species from Brazil's south, southeast and north (Amazon) regions

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## ARTICLE INFO

### Keywords:

Mercury  
Honey  
Native bees  
Brazil

## ABSTRACT

Food produced in regions with intense industrial or mining activity can be contaminated with heavy metals, including mercury (Hg). Human exposure to this metal can cause serious health problems. The aim of this study was to quantify and compare the levels of total Hg (THg) in samples of honey (n= 44) produced by five species of stingless bees, as well as to compare the THg values among the collection regions. Samples were obtained from three Brazilian states, one each in the South, Southeast and North regions (respectively Paraná, São Paulo and Rondônia, the last in the Amazon rainforest). The average THg level in the honey samples was 0.00060 mg.Kg<sup>-1</sup>. The results also indicated a significant difference among the collection places. The high variability of the THg values in the honey samples might have been related to the territory foraged by the bees and the types of plants visited, but not the species or their behavior. The divergence among the average levels found for the different collection areas could have been a reflection of the types of predominant industrial activities in the analyzed regions.

## 1. Introduction

Honey is produced naturally by bees from the nectar collected from flowers and/or secretions from the living parts of plants. These secretions, after being dehydrated, are stored in honeycombs for natural maturation (Nicewicz et al., 2021). The composition and chemical properties of honey depend on the botanical nature of the origin of the néctar (Li et al., 2022).

According to Abdullah et al. (2020), *Apis mellifera* L. is the predominant bee species that produces honey, although it is also produced by native bees (Hymenoptera: Apidae), called stingless bees due to their atrophied stingers. These bee species are commonly found in tropical forests and subtropical regions of the world. The honey produced by stingless bees is used in folk medicine to treat lung diseases, inappetence and eye infections, due to its immunological, anti-inflammatory, analgesic and sedative effects (Costa et al., 2019). In general, it is classified as a clean and healthful natural substance. However, the majority of

honeys are produced in regions that are possibly polluted by different sources of contamination, thus altering the quality (Di Noi et al., 2021).

According to Gekièrè et al. (2023), bees are among the organisms considered to be bioindicators of environmental contamination and pollution. These organisms can reflect the environmental conditions of a given locale, and bees typically visit a larger territory than the majority of terrestrial organisms (Zarić et al., 2022). Therefore, analysis of bees is increasingly being used for biomonitoring of atmospheric pollution and the impacts generated by various industrial activities (Shi et al., 2023; Taylor et al., 2023).

As described by Guo et al. (2020), analysis of honey, and to a lesser extent bees themselves, is also used to gather information about the habitats in which bees live. The authors attributed this to the fact that when bees fly in search of food, they contact the air, soil and water of the respective foraging territories, and return to the hive with similar concentrations of the toxic metals present in these areas. In another study (Taylor et al., 2023), the authors stated that the chemical composition of

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honey reflect the quantities of substances existing in the entire bee foraging region, so that honey can serve as a good biological indicator.

The contamination of honey and other bee products by heavy metals and other toxic elements may be due to the presence of these chemical elements in the environment in which bees produce these products (Díaz et al., 2023).

Of the heavy metals, Hg deserves special attention. According to Oliveira et al. (2018), when emitted to the atmosphere by natural soil erosion and volcanic activity and/or anthropogenic sources (industrial emissions, gold mining, forest fires), this metal is converted into inorganic (Mercury vapor) and organic mercury (methylmercury).

In both chemical forms, Hg when present in food poses risks to human health and has a cumulative effect on the body. That situation demonstrates the importance of determining of Hg in foods such as honey, not only for monitoring environmental contamination, but also for toxicological quality control of this food. Oliveira et al. (2018) further added that all heavy metals present in honey at high concentrations are considered to be potentially toxic to humans, and the risks are greater with higher honey consumption.

According to the World Health Organization (2017), exposure to high Hg levels can cause various adverse effects in the human body, affecting the central nervous, digestive and immunological systems. In turn, according to the European Food Safety Authority (2012), the monitoring of the levels of this metal in environmental and food samples is essential to assess the health risks and take the proper actions to protect the environment and human health. It is worth mentioning that fish is the most representative and studied food worldwide with regard to mercury concentrations, mainly due to the high organic mercury (methylmercury) content it generally has. This fact demonstrates the importance of carrying out studies of mercury concentrations in other foods, such as honey.

Therefore, the objective of this study was to assess the levels of THg in honey samples from different stingless bee species in the Brazilian states of Rondônia, Paraná and São Paulo, belonging to the North (mainly Amazon), South and Southeast regions, respectively, and to compare the levels of THg in the honey samples, broken down by bee species.

## 2. Materials and methods

### 2.1. Collection of samples and areas analyzed

We analyzed 44 honey samples from five stingless bee species, namely: *Tetragonisca angustula* (Latreille, 1811) [n=26]; *Scaptotrigona bipunctata* (Lepeletier, 1836) [n=8]; *Melipona quadrifasciata* (Lepeletier, 1836) [n=6]; *Melipona bicolor schencki* (Lepeletier, 1836) [n=3]; and *Tetragonisca weyrauchi* (Schwarz, 1943) [n=1], obtained from meliponaries in 13 municipalities in the state of Paraná, three municipalities in Rondônia and one municipality in São Paulo. The samples were gathered in line with the best honey collection practices so as to avoid direct or cross-contamination of the samples.

After collection, the honey samples were placed in sterilized polypropylene pots and stored under refrigeration at a temperature of approximately  $-4^{\circ}\text{C}$  in the dark. The samples were then transported in ice chests to the Wolfgang C. Pfeiffer Environmental Biochemistry Laboratory of Federal University of Rondônia, in Porto Velho, where they were stored in a freezer ( $-18^{\circ}\text{C}$ ) until measurement of THg.

### 2.2. Analytic quality control

All glassware was previously washed in 5% (v/v)  $\text{HNO}_3$  (Merck, Darmstadt, Germany) and rinsed with ultra-pure water (Milli-Q Plus, Millipore, Bedford, MA, USA). Quality control was done using blanks and samples in duplicate. A certified reference sample (DORM-4) (Fish Protein Certified Reference Material for Trace Metals), supplied by the National Research Council of Canada (NRCC, Ottawa) was digested and

analyzed under the same conditions as the samples. Furthermore, a fresh certified sample was prepared for each day of analysis.

A standard solution of certified mercury (Hg) at concentration of  $1000\text{ mg}\cdot\text{L}^{-1}$  (Merck, Darmstadt, Germany) was used to prepare a stock solution of  $2\text{ mg}\cdot\text{L}^{-1}$  Hg in 5%  $\text{HNO}_3$  (v/v). The calibration curve of the standard solutions prepared was based on concentrations of: 0.0, 1.0, 2.0, 5.0, 8.0 and  $10.0\text{ }\mu\text{g}\cdot\text{L}^{-1}$  in 5%  $\text{HNO}_3$  (v/v).

The limit of detection (LOD) for measurement of THg was calculated as  $\text{LOD} = 3.3 \times (\text{s}/\text{S})$ , where “s” is the standard deviation of the blanks and “S” is the angular coefficient ( $x$  in  $y = ax + b$ ) of the calibration curve. The correlation coefficient ( $R^2$ ) was calculated for each analysis day, and all values were greater than 0.999, and the equation of the straight line obtained was  $y = 0.0233x - 0.0009$ . We considered recovery percentages between 80% and 120% for the certified reference sample (DORM-4) as proof of the good accuracy of the analytical method employed to quantify THg. The mean recovery obtained from DORM-4 digestion was  $92.84 \pm 2.23\%$ . The analytical quality control results are presented in Table 1 and were in good concordance with the certified values. Also, the recovery percentage was satisfactory, indicating high accuracy of the analytical technique employed.

### 2.3. Chemical digestion of the samples and quantification of THg

The chemical digestion of samples was adapted from the method described by Maragou et al. (2016), with the addition of 5.0 mL  $\text{HNO}_3$  (65% w/w) and 2.5 mL  $\text{H}_2\text{O}_2$  (30 – 32% w/w, Merck, Darmstadt, Germany). This procedure was realized by open digestion in digestion tubes. For this purpose, approximately 1.0 g (wet weight) of each sample was weighed in duplicate, separately on an analytical balance (Shimadzu AY220, Kyoto, Japan) and added to the digestion tubes. The two tubes for each sample were placed in a digestion block (Tecnal 007 A, Piracicaba, São Paulo, Brazil) for 1 h at  $60^{\circ}\text{C}$  and then cooled to room temperature. After that, the samples were transferred again to the digester block. Then, were added 4.0 mL of 5% (w/v)  $\text{KMnO}_4$  (Merck, Darmstadt, Germany) and of tubes were heated again at  $60^{\circ}\text{C}$  for 30 minutes and then cooled to room temperature for 12 h and then titrated with drops of a double solution of 12% (w/v)  $\text{NH}_2\text{OH}\cdot\text{HCl}$  (Merck, Darmstadt, Germany) and 12% (w/v)  $\text{NaCl}$  (Merck, Darmstadt, Germany) and measured up to 10 mL with ultrapure water.

A cold vapor atomic absorption spectrometer (CVAAS) model FIMS-400 (PerkinElmer, Waltham, MA, USA) was used for the determination of the THg AT a wavelength of 254 nm. For the cold vapor generation of mercury, we used a double solution of 0.2% (w/v)  $\text{NaBH}_4$  (Sigma-Aldrich, Saint Louis, MO, USA) and 0.05% (w/v)  $\text{NaOH}$  (Vetec, Duque de Caxias, RJ, Brazil) as reduction agent and a solution 3% (v/v)  $\text{HCl}$  (Merck, Darmstadt, Germany). Argon gas with 99.999% purity (Oxiporto, Porto Velho, Brazil) was used for the THg determination.

### 2.4. Statistical analysis

The statistical tests were carried out with the R software (R Core Team, 2023) of the European Environment Agency, authorized by a General Public License and downloaded from the site [www.r-project.org](http://www.r-project.org). The software packages used were readxl, ggplot2, Rmisc, tidyr, FSA, PMCMR, Hmisc, boot, gridExtra and RColorBrewer. A significance level of 5% ( $\alpha = 0.05$ ) was considered. The statistical tests were performed by the nonparametric bootstrap method, with simulation of 100,000

**Table 1**  
Results of analysis of the certified reference materials.

Matrix	Certified sample	Reference value ( $\text{mg}\cdot\text{kg}^{-1}$ )	Observed value ( $\text{mg}\cdot\text{kg}^{-1}$ )	Recovery (%)	LDT ( $\text{mg}\cdot\text{kg}^{-1}$ )
Honey	DORM-4	0.412	$0.382 \pm 0.01$	$92.84 \pm 2.23$	0.000001

resamplings from the stock sample. This method was used because the number of samples (n) collected in the state of São Paulo was much lower than those collected in Rondônia and Paraná. For this reason, it was not possible to be sure of the probabilistic distribution of the population. In such a situation, this method is a good option since it allows working with a bootstrap distribution (larger distribution) for the sample distribution.

The accelerated bias correction (CBa) method allows the mean confidence interval (CI) to be asymmetric, mainly when the data have strong asymmetry in relation to the medians. Hence, for the comparisons among three or more samples (from distinct states and bee species), the CI values were calculated by the nonparametric bootstrap method via CBa to assure more reliable results, especially for not requiring any probabilistic assumption in the presence of the small number of samples (n) collected in a determined state in comparison with the other two, with the same logic applying to the comparison among the bee species. Both tests are recommended when the data are not normally distributed, so they are nonparametric tests. The statistical treatment of the data was in line with that carried out by Oliveira et al. (2018), and as also described by Montgomery (2017).

3. Results and discussion

3.1. Comparison of the levels of THg in the honey samples by collection state

Fig. 1 presents the comparison of the distribution of THg levels in the honey samples by state.

A large variability was observed between THg values in honeys from the different Brazilian states evaluated. This correlation between sample size and variability is acknowledged as a common statistical principle, where larger sample sizes are more likely to encompass a broader range of values, thus resulting in increased variability of values obtained and vice versa (Montgomery, 2017).

The boxplot clearly shows the increased variability based on the greater length of the whiskers that extend from each end of the box. Based on the interpretation of Oliveira et al. (2018), higher variability of the concentrations of THg among samples of honey collected in a single state are associated with smaller amplitudes between the minimum and maximum values obtained for that state.

The confidence intervals of the means (CI) of THg were found

between the samples collected in Rondônia and Paraná. The same occurred between the samples from São Paulo and Paraná (Fig. 1), suggesting no statistical difference of the levels of THg from honey samples collected in the states in which the CI of the means were found. This hypothesis was proved by the difference of means tests, as shown in Table 2, which presents the statistical treatment of the average levels of THg in the honey samples collected in Rondônia, Paraná and São Paulo, as well as the number of samples (n) and the minimum and maximum values found for each state.

All the samples had THg levels higher than the LDT ( $> 0.000001 \text{ mg. kg}^{-1}$ ). The statistical treatment by collection state showed that the honey samples collected in Rondônia and Paraná were equal to each other ( $p \geq 0.05$ ), with the greatest mean values of THg observed in this study ( $p \leq 0.05$ ). Our initial hypothesis was not confirmed, since we expected that honey samples from Rondônia alone would account for the high average concentration of THg among the states evaluated. This was based on the fact it is located almost entirely in the Amazon biome, recognized globally for having high levels of various chemical forms of Hg present in its biotic and abiotic compartments (Oliveira et al., 2018). That expectation was probably not borne out because the samples were not collected in municipalities near the region of the Madeira River, where high levels of Hg have been reported in different environmental matrices, due mainly to the mining of gold in the region for more than four decades. The association of gold with mercury is due to the use of the latter metal for separation of gold from artisanal placer mining sediments (Oliveira et al., 2018).

The samples obtained from meliponaries in the state of São Paulo had the lowest average values of THg ( $p \leq 0.05$ ), but this did not statistically

Table 2  
Descriptive and discriminative statistics for the concentration of THg in samples of honey from stingless bees, by collection state.

States	n	Min.	Max.	*Mean $\pm$ SD	Test of means
Rondônia	11	0.00047	0.00121	0.00073 $\pm$ 0.00006	A
Paraná	30	0.00012	0.00113	0.00064 $\pm$ 0.00004	AB
São Paulo	03	0.00028	0.00049	0.00042 $\pm$ 0.00005	B

n: number of samples. \*Average THg concentration in mg.kg-1 from 4 replications (2 aliquots of each sample and these aliquots were quantified twice each, therefore it is the average of 4 repetitions. SD: standard deviation. Min. and Max.: minimum and maximum values, expressed in mg.kg-1.

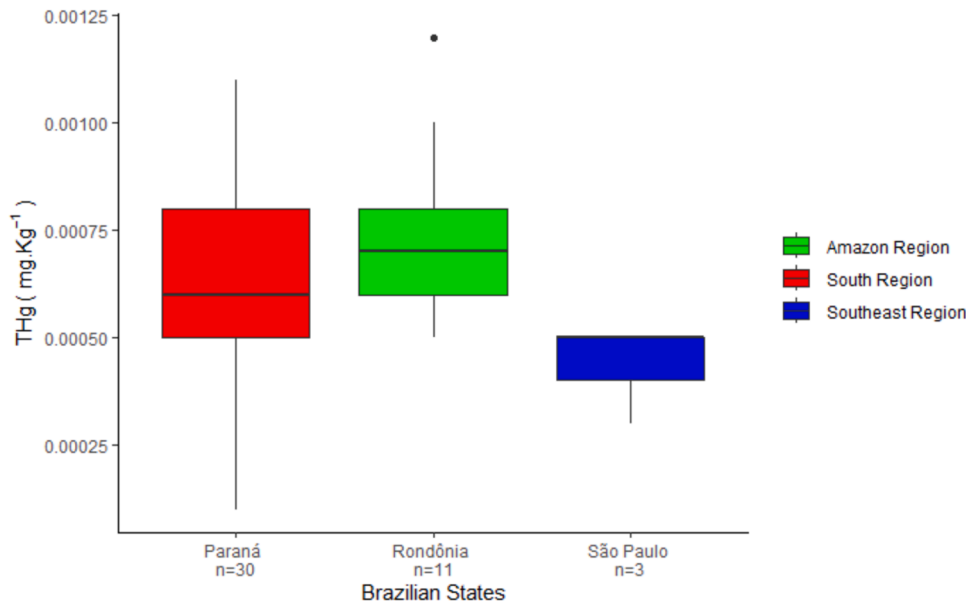


Fig. 1. Distribution of THg concentrations in honey samples from three Brazilian states.

differ ( $p \geq 0.05$ ) from the mean of the samples collected in Paraná.

We stress that we found very few studies of Hg specifically in honey samples despite a long and careful search in the literature. The most numerous studies have involved the use of bees as indicators of contamination and pollution in various regions of the world.

Of the works found involving evaluation of Hg in honey samples, only three came from Brazilian researchers (Silveira-Júnior et al., 2022; Vieira et al., 2014; Sodr  et al., 2007), and the samples analyzed in those studies came from the Northeast and Southeast regions. Thus, this study is groundbreaking by evaluating the concentrations of Hg in honey samples from a state in the South region (Paraná), as well as samples from Rond nia, located in the North region, specifically in the Amazon biome. Of these studies evaluating Hg in Brazilian honey samples, none involved honey from stingless bees, which also indicates the importance of our findings for the country.

Despite the scarcity of Brazilian studies and also international ones that have investigated the Hg levels in honey samples, some research groups (D az et al., 2023; Smith & Weis, 2022; Astolfi et al., 2021; Cheng et al., 2019) have reported that bees are good indicators of contamination in their foraging territory by other metals that are also toxic to human health, among them lead (Pb), chromium (Cr), cadmium (Cd) and arsenic (As). However, it is worth highlighting that although honey was has been used to monitor environmental contamination in some studies, it may not represent the real levels of pollution and contamination of the environment in which it was produced.

However, it is necessary to point out that of the toxic trace elements classified as heavy metals, Hg is the most relevant for its possible negative effects on health, mainly when reaching the central nervous system, where it can irreversibly impair various bodily functions (Oliveira et al., 2018). Therefore, this metal has been widely quantified in different foods in the world, but with only rare analysis of honey from stingless bees. In this respect, in the literature consulted we found only one study investigating heavy metals in honey from native bee species. That study was conducted in China and recently published (Shi et al., 2023), but the species were different from those we investigated and Hg was not among the metals evaluated. This fact also indicates the originality and merit of the data reported here, at the global level.

Of the foods studied in the world regarding Hg concentrations, fish is the most representative. The reason is that this food typically has a high level of organic Hg (methylmercury) in comparison with other types of foods. The presence of Hg in its most toxic form in fish can mainly be attributed to the fact that rivers, lakes and lagoons provide physical-chemical and microbiological conditions necessary to convert inorganic Hg into the organic form (Resci & C lia, 2023).

Nevertheless, Hg has strong affinity with humic substances, so when present in the soil it can be easily absorbed by plants. In a recent pioneering study in Brazil, Oliveira et al. (2018) found THg ( $0.0025\text{--}0.0086\text{ mg.kg}^{-1}$ ) in samples of yellow yam (*Dioscorea cayenensis*), a tuber particularly consumed by indigenous people of the Tupari ethnic group in the state of Rond nia (Brazilian Amazon), as well as in soil samples ( $0.042\text{--}0.083\text{ mg.kg}^{-1}$ ) from fields of this tuber and the other food plants studied (*Musa x paradis faca*, *Zea mays*, *Ipomoea batatas*, *Discorea trifida* and *Manihot esculenta*). The researchers attributed these results to the previously proven presence of Hg in the Amazon region.

Ali and Al-Qahtani (2012) stated that contamination of plant foods by metals should not be underestimated, since this source can increase levels of human exposure to these toxic elements. In this respect, the Hg released from natural and anthropic sources can be absorbed and accumulated in plants and also substances based on plants, such as honey, which can be contaminated by this highly toxic metal. According to Cunningham et al. (2022), bees come into contact with plants, nectar, pollen, sap or water and soil during foraging and can take these substances adhered to their bodies back to the hive, thus causing contamination of honey by Hg. Perugini et al. (2011) had previously explained that the heavy metals present in the atmosphere can also be deposited

directly on the bodies of bees and be released in the hive after foraging when the bees furiously beat their wings. The authors also evaluated bees (*Apis mellifera*) from various places in central Italy, including polluted urban areas and unpolluted rural ones, acting as possible bio-monitors of pollution. Of the heavy metals adhered to the bodies of the bees, Hg was not detected in that study. The authors attributed this finding to the fact that the metal was probably not present in the bees' foraging territories, because if it had been present, at least vestiges would have been detected, as was the case of the other metals analyzed (Cr, Cd and Pb).

More recently, Murashova et al. (2019) also investigated the presence of Hg on the bodies of *A. mellifera* bees sampled in the region of Ryazan, Russia. Unlike Perugini et al. (2011), these authors only did not find Hg on the bodies of young bees (up the 3 days of age), and suggested that this metal accumulates with time on bees. In turn, Waiker et al. (2022) detected that the accumulation of Hg on bees tended to increase with the degree of urbanization of their foraging territory, finding that the levels of Hg in honey samples collected from bees in 10 different locales in the United States were highest in heavily urbanized areas, intermediate in suburban areas and lowest in rural areas.

The contamination of other bee products by heavy metals, including Hg, has also been analyzed. Joveti  et al. (2018) detected the presence of this metal on the order of  $0.051\text{--}0.519\text{ mg.kg}^{-1}$  in pollen collected and stored by bees in an experimental apiary at the University of Belgrade in Serbia. The researchers also found Hg concentrations ranging from  $0.018$  to  $0.061\text{ mg.kg}^{-1}$  in samples of nectar from the flowers visited by the bees. Gajger et al. (2016) analyzed the presence of As, Cd, Pb and Hg in beeswax and attributed the high levels of Hg ( $0.29\text{--}1.46\text{ mg.kg}^{-1}$ ) to the modern apiculture practice of intensively recycling this material during decades, so that the wax accumulates heavy metals with time.

For these reasons and findings, scientific interest has increased regarding the presence of various trace elements, including Hg, in honeys. Nevertheless, Hg has been the least investigated metal in this respect.

According to Adugna et al. (2020), the results of the scarce studies carried out indicate that multifloral honeys are good indicators of environmental contamination by metals in reasonably large areas. In this regard, Zari  et al. (2022) reported that bees collect nectar from plants in areas up to  $100\text{ Km}^2$ . The large foraging territory of bees was also corroborated by Goretti et al. (2020), who evaluated the bee contamination index (BCI) based on the bioaccumulation of metals in honey of 35 locales in the central Italian region of Umbria.

In the present study, the Hg levels in the honey samples collected in the three Brazilian states were much lower than those found by Vieira et al. (2014) in 35 samples of honey (from *A. mellifera* bees) in different cities in Minas Gerais, a state in Brazil's Southeast region, with value reaching  $0.0025\text{ mg.kg}^{-1}$ . On the other hand, Sodr  et al. (2007) did not detect Hg when analyzing the presence of various trace metals in 38 samples of honey from the state of Pia  , in the Northeast region, also from *A. mellifera*. The same absence was reported by Silveira-J nior et al. (2022) in analyzing honey samples ( $n=21$ ) collected in the municipality of Caet  , located in the Serra Geral microregion of the state of Bahia, Northeast Brazil.

In international comparisons, the average levels of Hg in the honey samples from S o Paulo included in this study were near the minimum values reported by Girolametti et al. (2023) in honey from the Marche Region in Central Italy ( $0.0002\text{ mg.kg}^{-1}$ ) and by Oroian et al. (2016) in honey samples from Romania ( $0.00037\text{ mg.kg}^{-1}$ ). The study carried out by Girolametti et al. (2023) is particularly relevant because in addition to addressing honey as a bioindicator of environmental pollution by mercury, the authors also measured the corresponding hazard quotient (HQ) for human health related to the consumption of the honey samples they analyzed. They did not find any risk to human health through the consumption of the samples they analyzed. Considering the similarity of the values of THg found by these researchers and the state of S o Paulo included in this study, we suggest that the samples from this state also do

not represent a risk to human health.

Idoko et al. (2018) found average concentration of Hg in order 0.654 mg.kg<sup>-1</sup> in samples (n=10) collected in the villages of Brinin-Gwari Udawa and Brinin-Gwariof Kaduna in Nigeria, these values higher than those found in this study. Higher concentrations of Hg also were described (0.013–0.082 mg.kg<sup>-1</sup>) by Jovetić et al. (2018) in honey from central region of Zemun (Serbia).

Of the studies mentioned so far that evaluated honey among the bee substances, none evaluated the Hg levels in honey produced by stingless bees of the same species as we did. Of the works located, only that of Cheng et al. (2019) evaluated the honey of a stingless bee (*Heterotrigona itama*) from Malaysia, finding the presence of Hg along with other heavy metals (Cd, Pb, As and Sb) lower than 0.00001 mg.kg<sup>-1</sup> in all the samples evaluated.

3.2. Comparison of the THg levels in the honey samples by bee species

Fig. 2 depicts the comparison of the distribution of levels of THg in the honey samples by bee species.

Just as there was variability in mercury values during the comparison between states (Fig. 1), this was the case of the comparison between species. This high variability is also due to differences between sample quantities of each group (species), i.e., an obvious statistical consequence of the number of groups sampled.

Table 3 presents the statistical comparison of the average values of THg obtained for each bee species, along with the lowest and highest levels obtained for each species.

The honey from the species *T. angustula* and *S. bipunctata* had the highest average concentrations of THg among the species studied, without significant differences between them ( $p \geq 0.05$ ). *M. quadrifasciata* produced honey with the second highest average THg level, on the order of 0.00056 mg.kg<sup>-1</sup> ( $p \leq 0.005$ ), while the honey from *M. bicolor schencki* presented the lowest ( $p \leq 0.05$ ) average concentration in comparison with the samples from the other bee species.

Of the 26 honey samples from the species *T. angustula*, 10 were collected in Rondônia, 15 in Paraná and one in São Paulo. Of the eight samples produced by *S. bipunctata*, seven were from Paraná and one came from São Paulo. The fact that the majority of the samples from *T. angustula* came from Rondônia and Paraná while most of the samples from *S. bipunctata* came from Paraná can explain the greater average concentration THg in the honey samples from these species in

Table 3

Descriptive and discriminative statistics of the concentrations of THg in honey samples produced by different bee species.

Scientific name	N	Min.	Max.	*Mean $\pm$ SD	Test of means
<i>T. angustula</i>	26	0.00047	0.00121	0.00066 $\pm 0.00006$	A
<i>S. bipunctata</i>	8	0.00049	0.00098	0.00074 $\pm 0.00006$	A
<i>M. quadrifasciata</i>	6	0.00024	0.00083	0.00056 $\pm 0.00009$	B
<i>M. bicolor schencki</i>	3	0.00012	0.00061	0.00038 $\pm 0.00001$	C
<i>T. Weyrauchii</i>	1	-	-	0.00070	—

n: number of samples. \*Average concentration of THg in mg.kg<sup>-1</sup> from 4 replications (2 aliquots of each sample and these aliquots were quantified twice each, therefore it is the average of 4 repetitions. SD: standard deviation. Min. and Max.: minimum and maximum values, expressed in mg.kg<sup>-1</sup>. — Species dropped from the data for only having one sample (n=1). Means followed by the same letters in the column do not differ ( $p \geq 0.05$ ) from each other with regard to the mean quantity of THg in the honey samples produced by different bee species.

comparison with the others, but without a significant difference ( $p \geq 0.05$ ). Furthermore, these two states were responsible for the highest average THg level observed in this study, also without significant differences between each other ( $p \geq 0.05$ ), which also contributed to the finding that the two bee species accounted for the highest THg levels among the species, with statistically equal behavior between the two.

Another relevant observation was the smallest standard deviation found for the average THg levels in the honey samples produced by the *M. bicolor schencki*. This result can be explained by the fact that the three samples from this species came from a single meliponary in the municipality de São José dos Pinhais in Paraná. This hypothesis was based on the absence of differences in the foraging territory of the bees that produced these honeys, thus reducing the variability between the samples, and consequently lowering the standard deviation in comparison with the other species.

A single sample (n=1) of honey from *T. Weyrauchii* was collected, so that species was not included in the statistical analysis and comparison among the species. That sample had the lowest THg value obtained in this study (0.0007 mg.kg<sup>-1</sup>), although it was collected in the state of Rondônia (municipality of Alto Paraíso), the state that together with Paraná accounted for the highest mean value ( $p \leq 0.05$ ) of THg in the

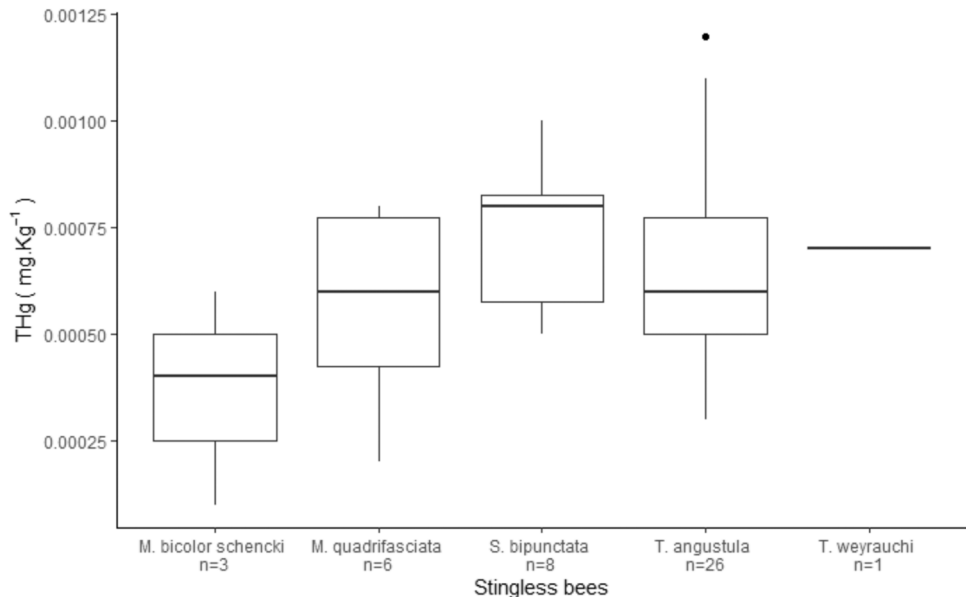


Fig. 2. THg concentration in honey samples from five stingless bee species in Brazil.

honey samples.

Oliveira et al. (2018) reported that the contamination of plants and plant foods by metal compounds, including Hg, can occur by two mechanisms – by uptake of these elements by the roots when present in the soil and/or water; or via atmospheric deposition, whereby the plant surfaces (leaves and flowers) retain particles of the metals carried in the air by means of gas exchange. According to these authors, the level of toxic elements accumulated in plants varies among plant species, since some plants absorb more metals than others in the same region or habitat. This assumption of a difference in the ability to absorb metals is supported by the findings of Bilandžić et al. (2014), who observed higher Hg levels in honey samples from chestnut trees (*C. sativa* Mill.) and lower levels in honey produced by bees visiting carob trees (*Ceratonia siliqua* L.), both in the same foraging area in Croatia.

Gaona et al. (2019) analyzed the floral preferences for collection of nectar and pollen by two stingless bee species (*Melipona mimetica* and *Scaptotrigona* sp.) in a tropical forest in Ecuador, and observed that although some bees had floral preferences, most visited all the sources of available nectar and pollen. Based on this premise and the findings of Ghosh et al. (2020), it can be reasonably assumed that the greater the variety of plants near the meliponary, the greater will be the chances of contamination of honey by heavy metals, since the greater will be the possible existence species that are more efficient in absorbing metals.

Scivico et al. (2022) evaluated the effects of the Covid-19 lockdown on the environmental pollution in the Campania region (Italy) by analyzing honeybees for the presence of 11 heavy metals, including Hg. The results showed that the confinement during the pandemic and consequent reduction of labor activities reduced the emissions of chemical pollutants in comparison with prior years, and also reported the efficiency of bees for environmental biomonitoring.

#### 4. Conclusion

It's possible that the high variability observed in this study for THg levels in honey samples from different bee species collected may be related to the habitats visited by these bees and/or the types of plants and flowers visited, but not with bees or their behavior. The reason is that the specific type of each species and also the behavior of bees were not evaluated in this study, and we also evaluated a very small number of samples.

For a more robust conclusion about the statement that stingless bees native to Brazil are good indicators of contamination and environmental pollution, it is necessary to carry out other studies with a larger number of samples and species, and also to collect samples in all regions of Brazil.

#### Funding

The source of funding for the research was the National Council for Scientific and Technological Development (CNPQ), through the Process 303450/2021–2 and 408160/2022–5.

#### CRediT authorship contribution statement

**Natália A. Campos:** Writing – original draft, Investigation, Data curation. **Gabriel H.A. Holanda:** Investigation, Data curation. **Maria C. N.N. Recktenvald:** Validation, Methodology, Investigation. **Walkimar A. Costa-Júnior:** Validation, Methodology, Investigation. **Ludimilla Ronqui:** Writing – original draft, Resources. **Rejane S. Parpinelli:** Resources. **Josiel D. Froelich:** Software, Formal analysis. **Wanderley R. Bastos:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Débora F. Oliveira:** Writing – review & editing, Project administration, Formal analysis, Data curation, Conceptualization.

#### Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

#### Data Availability

Data will be made available on request.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106084.

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