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Determination and human health risk assessment of mercury in honey from the Brazilian eastern amazon

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ABSTRACT

Honey has nutritional and therapeutic properties that bring various health benefits. Heavy metals are a major source of environmental pollution. The aim of this study was to determine mercury levels in honey samples from the Brazilian eastern amazon using a direct mercury analyzer (DMA) and human health risk assessment. The levels of Hg found in the samples ranged from 0.10 to 0.73 ng g^{-1}, except for 13 samples which were below the detection limit. The values estimates of average dietary exposure to total mercury in honey for adults were below the PTWI (4 µg kg^{-1}), thus presenting a low risk for the percentage of exposure, and the target hazard quotient (THQ) evaluation obtained values below 1. This study shown that Hg are accumulating in honey, presumably facilitated by dietary sources of the bees and direct environmental exposure.

1. Introduction

Honey is produced by bees from the nectar of flowers and extrafloral nectaries [1]. Besides being used as a replacement for sugar, honey is also employed as a natural medicine, possessing several herbal properties, with particular emphasis on expectorant, anti-inflammatory and antimicrobial actions [2]. Most of the honey commercialized in the world comes from the species *Apis mellifera*, known colloquially as honey bees. However, in tropical and subtropical regions there are more than 400 species of social bees, called stingless native bees, which can produce good quality honey. The honey of these species differs from the honeys produced by bees, mainly in water content (moisture) and acidity, making it a less dense and more susceptible to fermentation [3].

Honey has a complex composition and it is considered a natural sweetener [4]. Its composition depends on both the visited plants and the climatic and environmental conditions [5]. As the presence of toxic elements can be observed in its composition, honey shows itself as an indicator of environmental contamination [6]. Due to the environmental cycle, trace elements emanating from atmospheric and industrial pollution accumulate in the soil and affect the nearby ecosystem. Most of these elements are absorbed by the plants trough the root, and are incorporated in nectar and consequently to honey produced by bees. According to some authors, there are plants that accumulate mercury, in particular, bryophytes and vascular plants. These plants beyond accumulation or mercury can release it through their aerial parts. When these aerial parts fall to the ground it can also add mercury to the soil. In addition, mercury that may be accumulates in plants can be released into the environment during bushfires [7,8].

Among the toxic elements, mercury requires special attention because even at low concentrations it is highly toxic and can cause serious damage to human health, possessing neuro- and immunotoxic properties. Continuous exposure to Hg vapor causes irritations to the eyes, skin and mucous membranes, besides causing irreversible damage to the central nervous system, which can lead to permanent injuries such as limb paralysis. Its environmental importance is related to its high power for contamination of the environment and its high toxicity to living things [9].

Various toxicological evaluations of foods have been conducted based on risk indices for human health. Agencies and organizations, such as the Institute of Medicine of the National Academies, the US

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Environmental Protection Agency (US EPA) and the Joint Expert Committee on Food Additives of the Food and Agriculture Organization / World Health Organization (JECFA), provide guidelines for the intake of heavy metals, such as mercury. Risk assessment involves scientific analysis that results in qualitative or quantitative explanations of the likelihood of harm associated with exposure to hazardous chemicals. Some of the main indices used to assess health risk include estimated weekly intake (EWI), target hazard quotient (THQ), maximum safe consumption quantity (MSCQ) and carcinogenic risk (CR). [10–12]

Some studies can be found in the literature on the determination of metals in honey samples [13–17], but only one study in Brazil involving determination of mercury in honey by direct mercury analyzer (DMA) in the state of Minas Gerais [18]. On the other hand, no study was found on the determination of mercury in honey samples from the brazilian eastern amazon. Consequently, studies regarding the investigation of mercury in honey samples are promising.

The direct mercury analyzer has been used to determine mercury in different matrices [18–20]. The DMA working principle is based on the thermal decomposition, catalytic conversion, amalgamation and atomic absorption spectrometry, where the absorption intensity is measured at 254 nm.

Due to the increase in illegal mining in the Northern region from Brazil, this study aimed to determine the concentrations of Hg in honey samples from seven bee species (*Apis melifera, Scaptotrigona sp., Melipona fasciculata, Melipona flavolineata, Melipona seminigra pernigra, Frieseomellita longipes, and Tetragonisca angustula*) from the Brazilian eastern amazon by direct mercury analysis, in order to check whether mercury is accumulating in the honeys consumed by the population and also to assess the potential health risk of consuming this honeys.

2. Materials and methods

2.1. Instrumentation

A direct mercury analyzer DMA-80 Tricell (Milestone, Sorisole, Italy) was used in the determination of mercury in honey samples. Oxygen gas (99.99 %, White Martins, Pará, Brazil) was used in the DMA.

2.2. Reagents and solutions

A stock solution of 1000 mg L^{-1} Hg (in the form of mercury chloride) for atomic absorption (Sigma, USA) was used in the recovery tests to check the accuracy of the procedure by DMA. All dilutions were made using ultrapure water (resistivity 18.2 MΩ cm) obtained from a Synergy-UV water purification system (Millipore, Bedford, USA).

The certified reference material GBW 07605 tea (CRMs, Beijing, China) was also used for accuracy of the procedure by DMA.

2.3. Samples

Thirty-six honey samples were studied. The samples were obtained from apiaries located in different cities from state of Pará, Brazil. These honey samples obtained are a hybrid of *Apis mellifera* breeds resulting from the mixture of European races with African and other six species of native bees (Apidae, Meliponini) from the Amazon region, popularly known as stingless bees. The six species studied are the ones of higher frequency in the stingless beekeeping in eastern Amazon. All samples were supplied by Embrapa Amazônia Oriental. The honey samples were stored at −4 °C until further analysis.

2.4. Analytical procedure

Approximately 100 mg of each sample ($n = 3$) was weighed directly into a nickel boat and then placed in the autosampler of the DMA. The samples were automatically transported to the furnace, where they were dried and then thermally decomposed in a continuous flow of oxygen. The mercury vapors were concentrated in the amalgamator, a system made up of gold wires with a large surface area capable of accumulating the monoatomic mercury gas (Hg^0). The temperature of the amalgamator was then rapidly raised to 650 °C, releasing the mercury vapors. The mercury vapors were directed by the oxygen flow to the reading cells with varying optical paths. The choice of reading cell to be used was made automatically, depending on the amount of total mercury in the sample. Quantification was carried out using atomic absorption spectrometry. The wavelength used was 254 nm.The heating program for mercury determination in honey samples by DMA is shown in Table 1.

Due to the lack of honey certified reference material, the accuracy of the proposed method was evaluated using the analyte addition and recovery method according to Vieira et. al. (2014) [18]. Aliquots of 10 and 30 ng mL^{-1} Hg were added to a honey sample and determination of Hg by DMA was performed. The tea certified reference material (GBW 07605) also was used for verified the accuracy of the DMA analysis procedure.

2.5. Health risk assessment

2.5.1. Estimated weekly intake (EWI)

The estimated weekly intake (EWI) for mercury in honey is determined from the equation:

$$EWI = \frac{(C_{Hg} \times IR)}{BW}$$

Where, CHg and the concentration of mercury in the samples (μg g^{-1}), IR and the average food intake (150 g of honey per week) and BW and the average body weight (Kg), which in Brazil we have a BW average of 30 kg for a child and 70 kg for an adult. The EWI is an index for comparison with the provisional tolerable weekly intake index (PTWI), whose value has already been established by the joint FAO/WHO food additives expert committee (JECFA) [21]. The PTWI for inorganic mercury, which can be used to assess total mercury in foods, is 4.0 μg kg^{-1}.

The risk for mercury exposure can be calculated by the ratio of EWI to PTWI, using the following equation:

$$Risk_{Hg} = (\frac{EWI}{PTWI}) \times 100$$

2.5.2. Target hazard quotient (THQ)

The target hazard quotient (THQ) describes the non-carcinogenic health risk posed by exposure to the respective toxic element. For THQ below 1, non-carcinogenic health effects are not expected, for values greater than 1 there is the possibility of adverse health effects. The THQ index is calculated from the equation [22]:

$$THQ = \frac{(EF \times ED \times IR \times C_{Hg})}{(RD \times BW \times AT)} \times 10^{-3}$$

Where, EF and frequency of exposure (365 days per year), ED and duration of exposure, which corresponds to the average age of Brazilian adults (70 years), IR and average honey intake (g per day), C_{Hg} and the concentration of mercury in the food (μg g^{-1}), RD and the reference dose (0.0003 mg g^{-1} per day), BW and average body weight (70 kg), AT and

Table 1
Optimized heating program of the direct mercury analyzer.

Time (min:s)	Temperature (°C)	Step
00:00:30	25	Start
00:01:00	250	Ramp
00:01:00	250	Drying
00:01:30	650	Ramp
00:01:00	650	Decomposition
00:01:30	25	Cooling

the average time of consumption of the food (EF x ED).

2.6. Statistical analysis of data

For the statistical treatment of the results presented in this study, we used basic descriptive statistics (mean values and standard deviation). To test the statistic difference of means between the honey samples we conducted an Analysis of Variance (ANOVA) and the Fisher's least significant difference test (LSD test) for pairwise comparison were performed using Statistica Software 8.0 (StatSoft, Inc., Tulsa, USA) for data analysis. The significance level obtained for statistical analysis of data was $p < 0.05$.

3. Results and discussion

3.1. Detection limit (LOD) and quantification limit (LOQ)

LOD and LOQ were obtained to assess the sensitivity of the proposed procedure. Ten analytical repetitions of a honey sample with low mercury level were performed. The limit of detection was calculated as three times the value of the standard deviation of the ten measurements of the sample with low mercury concentration divided by the angular coefficient of the analytical curve. On the other hand, the limit of quantification was calculated as 10 times the value of the standard deviation of the ten measurements of the sample with low mercury concentration divided by the angular coefficient of the analytical curve were determined. The limit of detection and limit of quantification found were 0.01 ng/g and 0.03 ng/g Hg, respectively. The quantification limit found was 83 times smaller than the value obtained in studies with honey samples by Vieira et al. [18].

3.2. Validation method

The recovery obtained in the addition and recovery method for 10 and 30 ng/mL Hg were 103.3 % and 110.4 %, respectively. These percentages are in accordance with the recommendations of the AOAC [23], where the desired values are between 75 % and 120 %. The tea certified reference material (GBW07605) showed a Hg concentration of 0.013 mg/kg (90.0 % of recovery). This value found in GBW07605 agrees at a 95 % confidence level by *t*-test. The recovery obtained was acceptable and makes it possible to infer that the accuracy of the DMA measurement is adequate.

3.3. Determination of mercury in honey samples

Table 2 shows the levels of Hg obtained in honey samples from the Brazilian eastern amazon.

The results obtained shown that Hg are accumulating in honey. This could be due to dietary sources of the bees and direct environmental exposure.

The levels of Hg found in the samples were below the limit established by Brazilian legislation (500 ng/g). The honey sample from Belterra-PA (Sample 2) presented the highest mercury content (0.73 ± 0.06 ng g^{-1}) when compared to the other studied samples, but still very low compared to the limit established by Brazilian legislation. This level found in the municipality of Belterra can be due to the increase in mining activity in the Tapajós region. The Vigia honey sample (sample 20) from the Northeastern region from Pará also showed a high mercury value (0.45 ± 0.07). Depoi et al. [16] found higher levels of Hg (1.44–1.86 ng/g) in honeys from Brazil when compared to the levels obtained in this study. Akbari et al. [24] obtained a mercury content of 3030 ng/g in honey samples from Iran. Maggid et al. [25] found higher concentrations of mercury (0.72–31.69 ng/g) in honey from Tanzania when compared to the values obtained in the samples studied. The values obtained in the samples studied were lower than the values obtained by Vieira et al. [18] in 36 samples of honey from the Minas Gerais State, where all the samples had levels below the detection limit (< 2.5 ng/g). Toth et al. found high levels of mercury (0.008224–0.039892 mg kg^{-1}) in honey from Eastern Slovakia when compared with values obtained in this study [26]. In the honey samples from Argentina studied by Domínguez et. al. [22] were found high concentrations in the form of Hg^{2+} (69.3–113.5 μg kg^{-1}). Salama et. al. (2019) determined mercury levels (0.021–0.10 mg kg^{-1}) in honey samples from West of Libya [27]. Waiker et. al. (2022) found mercury levels (1.17 – 3.64 ng g^{-1}) in bee honeys from USA [28]. Fischer et. al. (2022) analyzed mercury concentration (0.01–1.71 μg/kg) in honeys collected on the territory of Poland [29]. Maragou et. al. (2016) found mercury content (< 0.05 μg g^{-1}) in honey from the Northern and Western part of Greece [30].

Table 2
The levels of Hg in honey samples (ng g^{-1} ± standard deviation, $n = 3$) from seven bee species.

Samples	Origin	Species	Hg (ng g^{-1} ± SD)
1	Belterra	*Scaptotrigona sp*	0.20 ± 0.01^{fg}
2	Belterra	*Scaptotrigona sp*	0.73 ± 0.06^{a}
3	Tracuateua	*Melipona fasciculata*	0.28 ± 0.03^{c}
4	Barcarena	*Melipona flavolineata*	0.28 ± 0.03^{c}
5	Tracuateua	*Melipona fasciculata*	0.26 ± 0.03^{d}
6	Igarapé-miri	*Melipona flavolineata*	0.18 ± 0.02^{h}
7	Igarapé-miri	*Melipona flavolineata*	0.10 ± 0.01^{i}
8	Igarapé-miri	*Apis mellifera*	< LOD
9	Igarapé-miri	*Melipona flavolineata*	0.10 ± 0.01^{i}
10	Igarapé-miri	*Melipona flavolineata*	< LOD*
11	Tracuateua	*Melipona fasciculata*	0.10 ± 0.01^{i}
12	Tracuateua	*Apis mellifera*	0.20 ± 0.01^{fg}
13	Castanhal	*Melipona fasciculata*	0.20 ± 0.01^{fg}
14	Castanhal	*Melipona flavolineata*	0.20 ± 0.01^{fg}
15	Igarapé-açu	*Melipona fasciculata*	0.22 ± 0.03^{e}
16	Igarapé-açu	*Apis mellifera*	< LOD*
17	Vigia	*Melipona flavolineata*	0.10 ± 0.01^{i}
18	St. Antônio do Tauá	*Melipona flavolineata*	< LOD*
19	St. Antônio do Tauá	*Melipona flavolineata*	0.10 ± 0.01^{i}
20	Vigia	*Apis mellifera*	0.45 ± 0.07^{b}
21	Vigia	*Melipona flavolineata*	< LOD*
22	Colares	*Melipona flavolineata*	< LOD*
23	Colares	*Apis mellifera*	0.28 ± 0.02^{c}
24	Colares	*Melipona flavolineata*	< LOD*
25	Colares	*Apis mellifera*	< LOD*
26	S. Caetano Odivelas	*Melipona fasciculata*	0.10 ± 0.01^{i}
27	S. Caetano Odivelas	*Melipona flavolineata*	0.10 ± 0.01^{i}
28	S. Caetano Odivelas	*Apis mellifera*	0.10 ± 0.01^{i}
29	Belterra	*Scaptotrigona sp*	< LOD*
30	Belterra	*Apis mellifera*	< LOD*
31	São João Pirabas	*Apis mellifera*	< LOD*
32	São João Pirabas	*Apis mellifera*	< LOD*
33	São João Pirabas	*Melipona fasciculata*	0.21 ± 0.02^{ef}
34	Belterra	*Tetragonisca angustula*	0.10 ± 0.01^{i}
35	Belterra	*Mellipona seminigra*	< LOD*
36	Belterra	*Frieseomellita varia*	0.28 ± 0.02^{c}

[a,b,c,d,e,f,g,h,i]Mean values with same lowercase letters do not differ statistically. Mean values with different lowercase letters differ statistically for the concentrations of Hg in honey samples from seven bee species (*Scaptotrigona sp., Melipona fasciculata, Melipona flavolineata, Frieseomellita varia, Apis mellifera, Tetragonisca angustula and Mellipona seminigra merrilae*) from the state of Pará, according least significant difference (LSD) test Fisher's ($p < 0.05$).*LOD = 0.01 ng g^{-1}

3.4. Health risk assessment

Results for the weekly estimate and the risk for this prevalence, and the target hazard quotient for the non-carcinogenic potential Table 3.

Table 3 can be observed in Table 3 that the values found for weekly honey intake are below the value established by JFECA for PTWI of adults. The THQ index was proposed by the USEPA [31], which established RD with a value of 0.0003 mg per kg per day [22]. THQ was also calculated using this RD value, and THQ values ranged from 0.0010 to 0.0074 (<1), as shown in Table 3, demonstrating a low health risk from

Table 3
Results for the weekly estimate and the risk for this prevalence, and the target hazard quotient, for the non-carcinogenic potential.

Samples	EWI		Risk (%)		THQ
	Child	Adult	Child	Adult	
Belterra	0.0010	0.0004	0.0250	0.0107	0.0020
Belterra	0.0037	0.0016	0.0913	0.0391	0.0074
Tracuateua	0.0014	0.0006	0.0350	0.0150	0.0029
Barcarena	0.0014	0.0006	0.0350	0.0150	0.0029
Tracuateua	0.0013	0.0006	0.0325	0.0139	0.0027
Igarapé-miri	0.0009	0.0004	0.0225	0.0096	0.0018
Igarapé-miri	0.0005	0.0002	0.0125	0.0054	0.0010
Igarapé-miri	0.0002	0.0001	0.0038	0.0016	0.0003
Igarapé-miri	0.0005	0.0002	0.0125	0.0054	0.0010
Igarapé-miri	0.0002	0.0001	0.0038	0.0016	0.0003
Tracuateua	0.0005	0.0002	0.0125	0.0054	0.0010
Tracuateua	0.0010	0.0004	0.0250	0.0107	0.0020
Castanhal	0.0010	0.0004	0.0250	0.0107	0.0020
Castanhal	0.0010	0.0004	0.0250	0.0107	0.0020
Igarapé-açu	0.0011	0.0005	0.0275	0.0118	0.0022
Igarapé-açu	0.0002	0.0001	0.0038	0.0016	0.0003
Vigia	0.0005	0.0002	0.0125	0.0054	0.0010
St. Antônio do Tauá	0.0002	0.0001	0.0038	0.0016	0.0003
St. Antônio do Tauá	0.0005	0.0002	0.0125	0.0054	0.0010
Vigia	0.0023	0.0010	0.0563	0.0241	0.0046
Vigia	0.0002	0.0001	0.0038	0.0016	0.0003
Colares	0.0002	0.0001	0.0038	0.0016	0.0003
Colares	0.0014	0.0006	0.0350	0.0150	0.0029
Colares	0.0002	0.0001	0.0038	0.0016	0.0003
Colares	0.0002	0.0001	0.0038	0.0016	0.0003
S. Caetano Odivelas	0.0005	0.0002	0.0125	0.0054	0.0010
S. Caetano Odivelas	0.0005	0.0002	0.0125	0.0054	0.0010
S. Caetano Odivelas	0.0005	0.0002	0.0125	0.0054	0.0010
Belterra	0.0002	0.0001	0.0038	0.0016	0.0003
Belterra	0.0002	0.0001	0.0038	0.0016	0.0003
São João Pirabas	0.0002	0.0001	0.0038	0.0016	0.0003
São João Pirabas	0.0002	0.0001	0.0038	0.0016	0.0003
São João Pirabas	0.0011	0.0005	0.0263	0.0113	0.0021
Belterra	0.0005	0.0002	0.0125	0.0054	0.0010
Belterra	0.0002	0.0001	0.0038	0.0016	0.0003
Belterra	0.0014	0.0006	0.0350	0.0150	0.0029

consuming these types of honey.

4. Conclusion

The results of this study do not give rise to any particular concern about mercury contamination in the honeys studied, and any risk of their being consumed by the population. However, future studies are needed to verify the chemical forms of mercury present in these honeys.

CRediT authorship contribution statement

Venturieri Giorgio C.: Writing – review & editing, Resources, Conceptualization. **Dantas Filho Heronides A.:** Writing – review & editing, Software, Data curation. **Batista Camila V.:** Methodology, Conceptualization. **Carvalho Fábio I. M.:** Writing – review & editing, Software, Data curation. **Cruz Allan da S.:** Validation, Formal analysis. **Borges Charles M.:** Writing – review & editing, Methodology, Data curation. **da Silva Brenda T. S.:** Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lopes Mônica da C.:** Validation, Formal analysis. **Dantas Kelly:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: KELLY DAS GRACAS FERNANDES DANTAS reports financial support was provided by Fundação Amazônia de Amparo a Estudos e Pesquisas do Pará (FAPESPA). KELLY DAS GRACAS FERNANDES DANTAS reports financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Allan da S. Cruz reports financial support was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Charles M. Borges reports financial support was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Camila V. Batista reports financial support was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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