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Assessment of metal contamination in *Tecoma stans* (L.) Kunth (Bignoniaceae) pollen samples from urban environments

Avaliação da contaminação por metais em amostras de pólen de *Tecoma stans* (L.) Kunth (Bignoniaceae) de ambientes urbanos

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ABSTRACT

Urbanization profoundly alters ecosystems, introducing various pollutants, including metals, which degrade environmental quality and pose risks to biodiversity, notably affecting pollinators like bees. Pollen, collected by bees during foraging, can serve as a bioindicator for assessing urban environmental quality, as it accumulates airborne and soil-derived contaminants. Studying metal concentrations in urban pollen is therefore critical, not only for monitoring pollution levels but also for understanding the potential toxicological impacts on bee populations, whose health is intrinsically linked to the quality of their food resources. This study aimed to assess the level of metal contamination in pollen samples of *Tecoma stans* (L.) Kunth, a plant species commonly found in urban settings and frequently visited by bees for foraging. Pollen samples were collected in the urban area of Uberlândia, MG, in 2023. Using spatial analysis of the city, sampling locations with distinct Bee Environmental Quality Index (BEQI) classifications — low, medium, and very high environmental quality — were selected to investigate the influence of environmental quality on metal concentrations. For this purpose, pollen samples from *T. stans* flowers were collected and analyzed at the designated sites, and the concentrations of aluminum (Al), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), and magnesium (Mg) were quantified using inductively coupled plasma optical emission spectrometry.

RESUMO

A urbanização altera profundamente os ecossistemas, introduzindo diversos poluentes, entre eles metais, que degradam a qualidade ambiental e representam riscos à biodiversidade, afetando notavelmente polinizadores como as abelhas. O pólen, coletado pelas abelhas durante o forrageamento, pode servir como bioindicador para avaliar a qualidade ambiental urbana, pois acumula contaminantes provenientes do ar e do solo. O estudo das concentrações de metais no pólen urbano é, portanto, essencial não apenas para monitorar os níveis de poluição, mas também para compreender os potenciais impactos toxicológicos sobre as populações de abelhas, cuja saúde está intrinsecamente ligada à qualidade de seus recursos alimentares. Este estudo teve como objetivo avaliar o nível de contaminação por metais em amostras de pólen de *Tecoma stans* (L.) Kunth, uma espécie vegetal comumente encontrada em ambientes urbanos e frequentemente visitada por abelhas durante o forrageamento. As amostras de pólen foram coletadas na área urbana de Uberlândia (MG), em 2023. Utilizando análise espacial da cidade, foram selecionados locais de amostragem com diferentes classificações do Índice de Qualidade Ambiental para Abelhas (Bee Environmental Quality Index — BEQI) — baixa, média e muito alta qualidade ambiental — a fim de investigar a influência da qualidade ambiental nas concentrações de metais. Para isso, amostras de pólen de flores de *T. stans* foram coletadas e analisadas nos locais designados, e as concentrações de alumínio (Al), cálcio (Ca), cádmio (Cd), cromo (Cr), cobre (Cu), ferro (Fe), manganês (Mn) e magnésio (Mg) foram quantificadas por espectrometria de emissão óptica com plasma indutivamente acoplado

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Statistical analysis, including PERMANOVA, indicated no significant differences in overall metal profiles across locations with varying BEQI levels, suggesting widespread metal presence regardless of the perceived environmental quality for bees. Principal component analysis and cluster analysis revealed a strong positive association among Fe, Cd, Cr, and Mn, suggesting common origins, while Ca and Cu showed a negative association. Our findings highlight that bees in urban areas are exposed to widespread metal contamination, even in environments classified as having very high BEQI.

Keywords: bioindicator; urban pollution; urban pollinators; Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES); Bee Environmental Quality Index (BEQI).

(ICP-OES). A análise estatística, incluindo PERMANOVA, não indicou diferenças significativas nos perfis gerais de metais entre locais com diferentes níveis de BEQI, sugerindo a presença generalizada de metais, independentemente da qualidade ambiental percebida para as abelhas. A análise de componentes principais (PCA) e a análise de agrupamento revelaram forte associação positiva entre Fe, Cd, Cr e Mn, sugerindo origens comuns, enquanto Ca e Cu apresentaram correlação negativa. Nossos resultados destacam que as abelhas em áreas urbanas estão expostas à contaminação generalizada por metais, mesmo em ambientes classificados como de qualidade ambiental muito alta segundo o BEQI.

Palavras-chave: bioindicador; poluição urbana; polinizadores urbanos; Espectrometria de emissão óptica com plasma indutivamente acoplado (ICP-OES); Índice de Qualidade Ambiental das Abelhas (IQAA).

Introduction

Changes in land use and land cover directly impact biodiversity and the maintenance of ecosystem services, with urbanization serving as a clear example. The urban environment can be understood as a dynamic system, wherein alterations in ecosystem flows may result in increased or decreased interactions, the disappearance of certain flows, or the emergence of new ones (Belem, 2020). In cities, various sources of atmospheric pollution, such as industries and motor vehicles, release metals that degrade environmental quality, affecting all living organisms, including bees.

Bees are essential pollinators in both natural and urbanized environments (Ayer and Rehan, 2021). Studies have shown that urban areas can serve as refuges for many bee species and that well-managed green spaces in these settings can support biodiversity and function as hotspots for the pollination services provided by these insects (Theodorou et al., 2020; Ayer and Rehan, 2021). However, in urban environments, bees — like other animals — are exposed to contaminants through multiple routes, including ingestion of contaminated food, direct contact with airborne particles, and consumption of water polluted with heavy metals or industrial waste (Gekière et al., 2023). Regarding food ingestion, studies from different regions confirm that urbanization can directly affect the quality of pollen and nectar consumed by bees, particularly through the biosorption and bioaccumulation of metals (Kalbande et al., 2008; Morgano et al., 2010; Nascimento et al., 2018; Scott and Gardiner, 2024; Mousa et al., 2025).

Pollen collected by bees for brood nourishment can serve as a bioindicator for monitoring atmospheric pollution (Şeker et al., 2022). In urban environments, rainwater can become contaminated as it passes through the atmosphere and over various surfaces, potentially transporting pollutants to the soil. These contaminants may include industrial residues and automobile-derived metals such as zinc (Zn), copper (Cu), boron (Br), lead (Pb), chromium (Cr), cobalt (Co), and barium (Ba), which infiltrate the soil during rainwater percolation (Belem, 2020). Consequently, metals can enter plants not only through surface deposition but also via root uptake from contaminated water.

Metals like copper (Cu), zinc (Zn), and iron (Fe) are considered essential for plant growth, but they can be toxic in elevated amounts (Rai et al., 2021). Moreover, elements such as cadmium (Cd) and chromium (Cr) are not naturally found in the environment and can be toxic and also accumulate in insects' bodies (Mielczarek and Wojciechowicz-Żytko, 2020). Sublethal effects on bees include impaired learning and memory performance (Burden et al., 2016; Monchanin et al., 2021; Kaila et al., 2023), as well as reduced colony and population growth — reflected in decreased brood cell construction and lower offspring survival in both social and solitary species (Moroń et al., 2013; Scott et al., 2022).

Depending on the metal, there may be no safe level of exposure for bees, even at sublethal doses. The metal pollutants, even at low levels — particularly arsenic (As) — effect the behavior and cognition of *Apis mellifera* bees (Monchanin et al., 2024). In *Bombus impatiens*, oral exposure to metals commonly present in urban environments — such as arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) — at both lethal and sublethal concentrations has been shown to increase brood mortality (Scott et al., 2022). It was verified that worker bees ingesting metal-contaminated resources exhibit reduced brood care behavior and reduced nest thermoregulation capacity, behaviors that directly affect brood survival.

Considering that the assessment of metal contaminants in pollen used by bees can serve as an important tool for biomonitoring environmental changes resulting from anthropogenic activities, the objective of this study was to evaluate metallic contamination in pollen collected by bees in urban areas. To evaluate the levels of metal contamination, we selected the melittophilous and exotic plant species *T. stans* due to its attractiveness to various pollinators and its wide use in urban landscaping as a proven floral resource. We start from the hypothesis that pollen collected by bees in urban areas presents different concentrations of metallic contaminants, and these concentrations vary depending on the environmental quality.

Materials and methods

Sample collection and preparation

The study was conducted in the urban area of Uberlândia, Minas Gerais (MG), Brazil. Six areas within the urban perimeter of Uberlândia, each approximately 1.5 km in diameter, were selected and surveyed until *Tecoma stans* specimens were located. These six areas were defined using a Bee Environmental Quality Index (BEQI) map as a reference (Figure 1). The BEQI was developed using Geographic Information Systems (GIS) and multi-criteria analysis to generate a map assessing environmental quality for bees. The process followed three main steps: (i) selection of themes/criteria — The criteria were chosen to meet the needs of the bees and the level of environmental quality that the location offers for them to have nesting and foraging conditions. Relevant factors were selected, including proximity to watercourses, slope, Land Surface Temperature, soil types, and land use/land cover; (ii) application of the Delphi Method (see Okoli and Pawlowski, 2004) — Experts reached, including professors, doctoral students, and postdoctoral fellows from several national and international research institutions, defined the weights for each criterion. To determine the weights, the Delphi method was employed with the participation of 15 specialists who completed a structured questionnaire. The panel was composed of professors, doctoral candidates, and postdoctoral researchers affiliated with institutions such as the Universidade Federal de Uberlândia, Instituto Federal do Triângulo Mineiro, Universidade Estadual de Goiás, Universidade do Estado de Mato Grosso, Universidade Estadual de Londrina, Universidade de São Paulo, the University of Lisbon, Instituto Superior de Agronomia, and one researcher from EMBRAPA Meio Ambiente. All participants were directly engaged in bee-related research. The consultation process was conducted in two rounds. In the first round, the experts ranked the importance of the environmental variables, and the results were consolidated into mean values and predominant trends. In the second round, each expert received the aggregated results and was invited to confirm or revise their previous responses. Anonymity was ensured throughout the process to prevent any potential influence among participants. This procedure adheres to the principles of the Delphi method, in which consensus is progressively achieved through successive anonymous consultations until the stabilization of opinions is observed (Moura, 2007; Minayo, 2014); and (iii) Multi-criteria analysis — Using QGIS 3.22.11 software (QGIS, 2024), data were processed, and weighted criteria were applied to generate the BEQI.

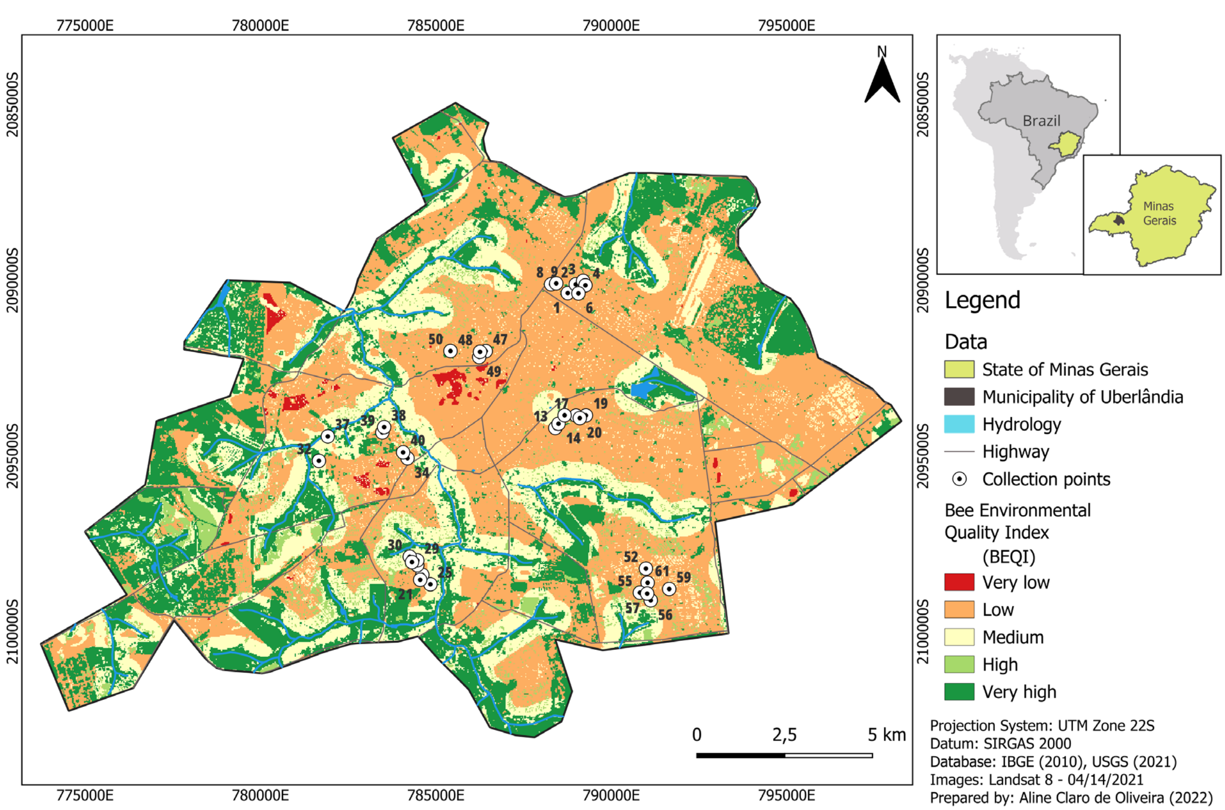

Figure 1 – Distribution of the 36 collection points of *Tecoma stans* flowers and buds across BEQI classes: low (n=19), medium (n=10), and very high (n=7).

The multi-criteria analysis was carried out using Weighted Linear Combination (WLC), where each criterion was multiplied by the assigned weight, generating a final layer that represents the BEQI, calculated by the QGIS raster calculator. Using the method of natural breaks (Jenks) in the SAGA GIS program, data from the multicriteria analysis were classified into five classes of environmental quality for bees, being: 1) very low; 2) low; 3) medium; 4) high; and 5) very high (Oliveira et al., in preparation). After calculating all indicators, the maps were processed through WLC, and the resulting continuous data were categorized into five classes of environmental quality for bees. The classification into very low, low, medium, high, and very high was performed using the natural breaks (Jenks) method in SAGA GIS, which optimizes class boundaries by minimizing intra-class variance and maximizing inter-class variance (Sartori et al., 2012). Thus, the class limits were not arbitrary but derived statistically from the distribution of the BEQI values. Table 1 shows the correspondence between pixel values and environmental quality classes.

The soil map was obtained from vector files made available by IBGE (2006) with a scale of 1:5,000,000 and was cut only for the study area. For calculation purposes, this layer was converted to raster format through the rasterization process. The land use and cover criteria directly reflect the availability of suitable habitats for bees. Areas with dense vegetation cover, such as urban forests and parks, provide food and nesting sites. In contrast, paved or built-up areas limit available resources and reduce environmental quality. The land use and cover map were prepared using bands 4, 5, and 6 of the OLI (Operational Land Imager) sensor on the Landsat 8 satellite acquired on the United States Geological Survey website, on 04/23/2021 for orbit/point 220/74, and 04/14/2021 for orbit/point 221/73. To obtain the false color composition, the composition 6R5G4B was made.

The collections of *T. stans* flowers were performed in March 2023, during the rainy season. In each area, samples were collected from 10 trees, totaling 60 trees (sampling points). From each tree, 120 flowers were gathered. The samples were stored in individual plastic bags for each tree and georeferenced using the free mobile app MAPinr. All plastic bags were labeled according to their corresponding georeferenced points. All glassware and utensils used for sample handling (Petri dishes, glass rods, and Falcon tubes) were previously washed with neutral soap and running water, followed by thorough rinsing with ultrapure water (18.2 MΩ·cm) obtained from a Gehaka purification system, to eliminate any possible residues or contaminants.

Table 1 – Bee Environmental Quality Index Class Grouping.

Pixel Value	BEQI
1	Very low
2	Low
3	Medium
4	High
5	Very high

To extract pollen from the stamens, 10 mL of ultrapure water was added to each Falcon tube, followed by manual agitation for 1 min. Excess plant material was removed with a glass rod, leaving only the pollen. The Falcon tubes were centrifuged at 3,000 RPM for 3 min, concentrating the pollen at the bottom of the tubes. The water was discarded, leaving only the pollen. Out of the 60 samples, 36 contained a sufficient quantity of pollen for chemical analysis.

Instrumentation and acid digestion of samples

Samples were digested following the acid digestion protocol described by Aldgini et al. (2019), with some adaptations due to the available pollen quantity in each collected sample. During the acid digestion step, 5 mL of HNO_3 was added to 0.05 g of pollen and heated in a digestion block for 20 min at 160°C. Subsequently, 1 mL of $HClO_4$ was added in three stages, with the mixture heated in the digestion block for 5 min at 148°C during each stage until the solution became clear. The solution was then filtered using a 0.22 µm filter and diluted with ultrapure water to a final volume of 25 mL in a volumetric flask. Out of the 60 samples, 36 contained a sufficient quantity of pollen for chemical analysis.

An analytical blank was prepared by performing the complete acid digestion procedure using only ultrapure water (18.2 MΩ·cm), obtained from a Gehaka ultra-purification system (São Paulo, Brazil), without the addition of pollen material, to serve as a control for the analytical process and account for any potential contamination. All blank readings were below the limits of detection for the analyzed elements, confirming the absence of measurable contamination.

Samples were analyzed using a dual-view inductively coupled plasma optical emission spectrometer (ICP-OES), model Avio 200 (PerkinElmer, Waltham, MA, USA). This technique was selected for its high sensitivity and accuracy in trace element detection. Analytical wavelengths (nm) employed for element quantification were as follows: aluminum (Al) (396.153), calcium (Ca) (317.933), cadmium (Cd) (228.802), chromium (Cr) (267.716), copper (Cu) (327.393), iron (Fe) (238.204), manganese (Mn) (257.610), and magnesium (Mg) (285.213). Spectral lines were chosen based on minimal spectral interferences, high signal-to-background ratios, and optimal precision.

ICP-OES operating conditions included: radio frequency power at 1500 W; plasma gas flow rate of 8.0 L min^{-1}; auxiliary gas flow rate of 0.2 L min^{-1}; nebulizer gas flow rate of 0.7 L min^{-1}; sample uptake rate of 1.0 mL min^{-1}; injector tube diameter of 2.0 mm; and signal integration time of 1 s. High-purity argon gas (99.99%) was used both for plasma generation and as the carrier gas.

Calibration parameters and analytical performance data for each element determined by ICP-OES are presented below. The limits of detection (LOD) and quantification (LOQ) were calculated according to ISO 11843-2:2000 (ISO, 2000) using six calibration levels (0, 0.01, 0.10, 0.50, 1.00, and 3.00 mg L^{-1}). Calibration curves were prepared using a certified multielement standard solution (G7V, Quimlab, Brazil; 100 mg L^{-1}, Lot F24J0239B) traceable to the National Institute of Standards and Technology (NIST).

The solution matrix consisted of 5% HNO_3 with traces of HF, and all standards were prepared gravimetrically using Class A volumetric flasks.

The LOD and LOQ were determined following the methodology established in ISO 11843-2:2000 (ISO, 2000), using the residual standard deviation of the regression ($s_Y/_x$) and the slope (b) of each calibration curve. All calibration curves showed excellent linearity ($R^2 \geq 0.998$), confirming the high sensitivity of the ICP-OES method for trace metal determination. Analytical blanks were included in each digestion batch and presented signals below detection limits, confirming the absence of measurable contamination. Detailed data of calibration curves, regression parameters, and blank measurements are provided in Table 2.

Statistical analysis

This study analyzed eight variables, Al, Ca, Cd, Cr, Cu, Fe, Mn, and Mg, across 36 sampling points located in six areas classified as low, medium, or very high environmental quality for bees, totaling 288 samples (Figure 1). Samples from twenty-three points were used to calibrate the acid digestion method. Consequently, the data matrix consisted of 36 objects (points representing sampling units) and eight descriptors (chemical elements).

Initially, cluster analysis was performed to group chemical elements with similar characteristics, aiming to identify patterns that could indicate potential common sources of contamination. This analysis classified the dataset into homogeneous groups based on their shared properties, which can reflect similarities in the origin, transport mechanisms, or environmental behavior of these metals. For example, grouping elements like Cd, Cr, and Mn may suggest that these pollutants derive from related anthropogenic sources, such as vehicular emissions or industrial activities, as reported in previous studies (Barbosa et al., 2021; Şeker et al., 2022). Cluster analysis condenses large datasets into an easily interpretable format, typically displayed as dendrograms (Silva et al., 2022). To investigate the relationships among the chemical elements and identify potential common sources, we first performed a hierarchical cluster analysis. This method groups variables based on the similarity of their concentration profiles across the samples, which can reflect shared origins, transport mechanisms, or environmental behaviors. The result is typically displayed as a dendrogram, where elements that cluster together are interpreted as being positively associated. The analysis was conducted using the pvclust package in R, which calculates approximately unbiased (AU) p-values and bootstrap probability (BP) values to assess the statistical significance of each cluster.

Next, to visualize the variation in metal composition among the different BEQI classes, we applied principal component analysis (PCA). PCA is an exploratory technique that reduces the dimensionality of complex datasets, summarizing the main patterns of variation in a few new variables called principal components. The scale function was used to standardize the data, ensuring that all variables had equal weight in the analysis. PCA is widely used across multiple disciplines, particularly in ecology, where it simplifies complex datasets, such as species distribution across locations and explanatory variables (Silva et al., 2022).

Table 2 – Calibration parameters and analytical performance of the ICP-OES method.

Element (nm)	Regression equation	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})
Ca (317.933)	$y=1.21\times10^6x - 20,143$	0.9992	0.082	0.212
Cd (228.802)	$y=8.77\times10^5x - 14,612$	0.9990	0.097	0.247
Cr (267.716)	$y=9.33\times10^5x - 17,045$	0.9989	0.109	0.273
Cu (327.393)	$y=1.02\times10^6x - 19,825$	0.9991	0.095	0.248
Fe (238.204)	$y=1.08\times10^6x - 18,976$	0.9993	0.103	0.261
Mn (257.610)	$y=1.15\times10^6x - 21,317$	0.9994	0.088	0.227
Mg (285.213)	$y=1.09\times10^6x - 20,812$	0.9992	0.091	0.239
Al (396.153)	$y=1.16\times10^6x - 19,197$	0.9995	0.115	0.289

Finally, to formally test the hypothesis that there were no significant differences in the overall metal profiles among the BEQI classes, we applied a permutational multivariate analysis of variance (PERMANOVA). This non-parametric statistical test is ideal for ecological data, as it compares the variation among groups to the variation within groups, providing a p-value to determine if the observed differences are statistically significant. This statistical technique tests multivariate hypotheses by comparing the abundance of different species in response to various treatments or environmental gradients (Silva et al., 2022).

All statistical analyses were conducted using the RStudio Team software.

Results

Comparing the eight chemical elements (Al, Ca, Cd, Cr, Cu, Fe, Mg, and Mn) detected in *T. stans* pollen samples, it was verified that Aluminum (Al) and Magnesium (Mg) exhibited the highest concentration (Table 3, Figure 2), while Cadmium (Cd) and Chromium (Cr), in contrast, were detected at considerably lower concentrations. A visual inspection of the boxplots suggests that, for most metals, variation within BEQI classes is substantial, with no clear trend of increasing or decreasing concentrations in relation to the BEQI. These observations align with the PERMANOVA results, which indicated no significant differences in overall metal profiles among BEQI classes. Detailed numerical data on the concentrations of each metal by sampling site and BEQI class are provided in Appendix A for further quantitative examination. All ICP-OES determinations were conducted under rigorous quality control procedures. Calibration curves were prepared from a certified multielement standard solution (G7V, Quimlab, Brazil; 100 mg L^{-1}, traceable to NIST), and analytical blanks measured below detection limits. These procedures ensured the accuracy and reliability of the reported metal concentrations. The LOD and LOQ determined for all analyzed elements were within the expected range for trace metal analysis by ICP-OES, confirming the sensitivity and robustness of the analytical method (Table 2).

Table 3 – Concentrations of metals (Al, Ca, Cd, Cr, Cu, Fe, Mg, and Mn, in mg/kg of pollen dry mass) in *Tecoma stans* pollen samples collected from sites with varying environmental quality for bees in Uberlândia, MG.

Collection points	BEQI	Al	Ca	Cd	Cr	Cu	Fe	Mg	Mn
1	Low	546.03	227.66	0	2.44	1.29	10.12	77.2	2.65
2	Low	89.26	233.67	0	1.95	1.32	14.58	135.68	1.17
3	Low	321.02	194.13	0	3.21	1.38	111.6	161.93	1.02
4	Low	352.43	199.54	0	3.23	68.03	10.64	26.8	2.85
6	Low	504.39	34.72	0.44	30.86	24.41	74.85	127.54	6.01
8	Low	298.23	163.00	0	2.10	1.11	7.91	24.67	0.80
9	Low	708.6	95.21	0.65	45.73	19.47	134.52	654.14	10.39
17	Low	173.66	167.80	0	4.54	1.26	24.69	37.12	1.43
19	Low	122.93	236.56	0	2.61	9.94	30	48.41	0.83
21	Low	504.7	67.38	0.31	33.07	22.17	517.78	556.53	12.76
26	Low	457.36	189.88	0	2.61	8.11	21.07	37.57	1.34
27	Low	1145.16	768.00	0	6.38	3.19	77.64	108.96	2.25
39	Low	227.48	148.93	0	1.74	0.79	8.78	31.44	0.40
47	Low	407.73	219.24	0	2.55	1.10	12.22	65.98	1.22
48	Low	639.91	103.62	0.78	61.38	44.31	239.48	504.4	85.95
49	Low	149.56	163.79	0	1.55	1.55	43.22	34.04	1.4
50	Low	414.79	66.53	0.43	26.95	18.51	445.24	606.02	13.65
57	Low	849.23	77.18	0.6	45.52	22.42	100.72	608.61	13.04
59	Low	1296.8	191.36	0	3.98	1.07	18.64	55.44	0.78
25	Medium	125.06	251.88	0	4.77	1.63	132.71	65.48	2.13
28	Medium	872.59	108.49	1.03	55.54	202.11	322.21	640.9	19.69
29	Medium	308.92	141.96	0	2.64	0.74	3.65	32.09	0.68
30	Medium	89.78	189.39	0	2.91	2.2	11.9	35.45	1.03
34	Medium	140.32	92.42	0	0.90	1.52	11.23	25.68	0.67
38	Medium	724.9	94.85	1.18	57.87	77.16	449.71	703.76	140.41
52	Medium	327.81	70.91	0.3	24.1	228.79	75.77	750.49	10.28
55	Medium	327.28	91.07	0.32	22.71	15.51	75.67	819	18.88
56	Medium	423.51	87.68	0.36	26.05	135.87	168.32	1055.15	23.79
61	Medium	482.15	77.00	0.45	30.97	97.45	149.01	575.00	16.31
13	Very high	138.88	381.63	0	4.78	1.15	52.72	103.79	1.81
14	Very high	266.96	92.86	0	1.62	0.64	3.19	21.08	0.49
16	Very high	307.02	68.44	0.31	23.75	429.48	117.77	585.62	10.87
20	Very high	140.92	177.33	0	1.92	1.01	22.83	42.31	0.96
32	Very high	584.00	120.53	1.02	33.72	45.63	128.3	620.27	18.90
37	Very high	19.45	557.41	0	4.77	2.45	105.61	187.70	2.91
40	Very high	619.93	83.44	0.69	50.02	34.73	184.39	521.74	15.90

The relationships among the metals are clarified by the cluster analysis and PCA. In the similarity dendrogram (Figure 3), the elements Fe, Cd, Cr, and Mn form a statistically significant cluster (AU>95%), indicating that their concentrations tend to co-vary across the samples. This suggests these metals may share common environmental sources or pathways.

PCA revealed that the first two components (PC1 and PC2) together explained 69.9% of the total variance in metal concentrations (Figure 4). The considerable overlap of the BEQI class ellipses and the small distances between their centroids indicate no clear separation in metal profiles among environmental quality classes. In the PCA biplot, Al, Fe, Cd, Cr, and Mn vectors were oriented similarly, indicating positive associations, while Ca and Cu vectors pointed in opposite directions, revealing a negative association. Whereas, aluminum (Al) showed no strong relationship with the other metals, suggesting distinct environmental behavior or sources. PERMANOVA results corroborated the PCA interpretation. The variance explained by the predictor variable (environmental quality) was approximately 9.4% (0.09426). This suggests that BEQI does not significantly influence overall variation in metal availability (p=0.1331).

Taken together, the PCA, PERMANOVA, and cluster analysis reinforce the interpretation that metal contamination is widespread across the urban landscape, regardless of BEQI classification and the positive association among the elements Fe, Cd, Cr, and Mn.

Discussion

The central finding of this study is the absence of a significant correlation between environmental quality for bees, as measured by the BEQI index, and metal contamination levels in *T. stans* pollen. The LOD and LOQ calculated for all analyzed metals (Table 2) were within the expected range for trace metal determination by ICP-OES, indicating adequate method sensitivity. Despite the overall accuracy and precision of the technique, some sources of uncertainty must be acknowledged. Possible analytical errors include matrix effects caused by residual organic components in pollen digests, instrumental drift, and minor spectral interferences inherent to complex environmental samples. However, the use of a certified multielement standard solution (Quimlab, traceable to NIST), procedural blanks, and rigorous calibration procedures minimized these potential biases, ensuring the reliability of the reported concentrations. PERMANOVA results confirm that contamination is widespread across the urban landscape of Uberlândia, affecting both low- and very high-quality areas.

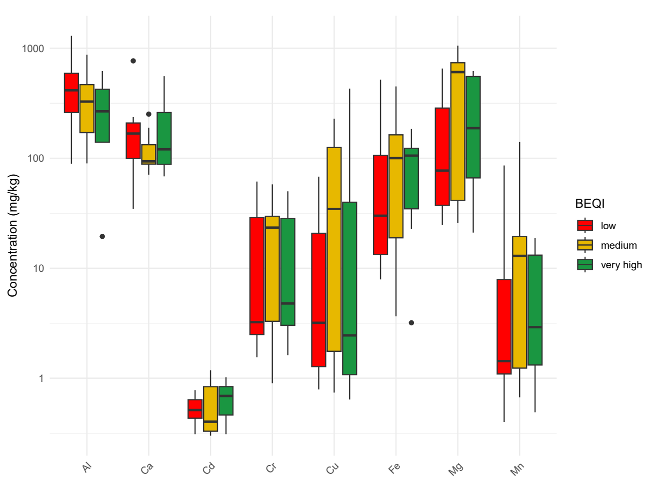

Figure 2 – Boxplots of metal concentrations (mg/kg) in *Tecoma stans* pollen across Bee Environmental Quality Index classes: low (red), medium (yellow), and very high (green). The plots display the median, interquartile range, data dispersion, and outliers for each element (Al, Ca, Cd, Cr, Cu, Fe, Mg, and Mn).

The PCA revealed a strong association among Fe, Cd, Cr, and Mn, which clustered in the same vector direction, indicating that these elements tend to vary together across sampling sites. In contrast, Ca and Cu vectors pointed in opposite directions, reflecting a negative association between these metals. Aluminum (Al) and magnesium (Mg) showed weak relationships with the other elements, suggesting distinct environmental behaviors or sources. The extensive overlap among the BEQI class ellipses, together with the short distances between centroids, indicates that metal concentration patterns do not differ substantially among environmental quality classes, reinforcing the idea of widespread contamination across the urban landscape. In addition, it is important to highlight that the points located in regions classified as having high environmental quality are predominately surrounded by sites with low and medium BEQI.

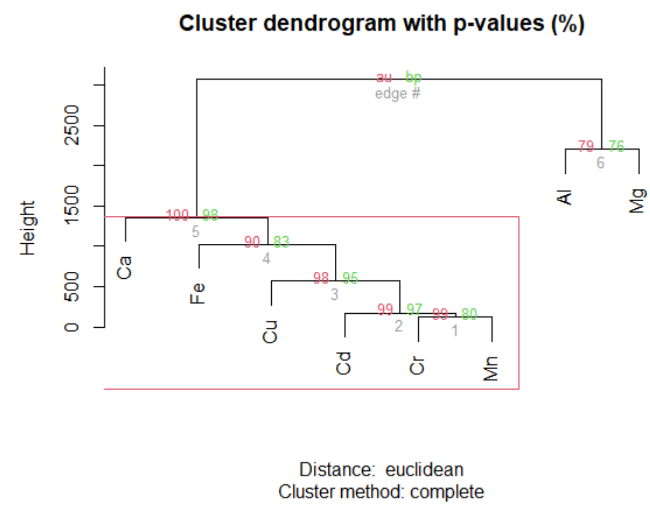

Figure 3 – Similarity dendrogram determined using the hierarchical clustering method with Euclidean distance. Values of Approximately Unbiased, showed in red, and Bootstrap Probability, showed in green.

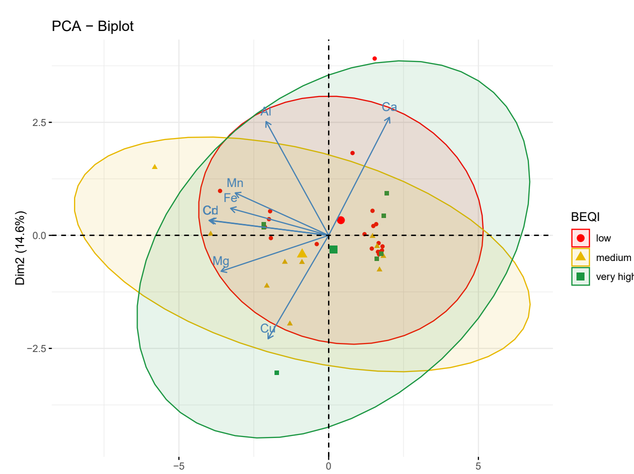

Figure 4 – Principal Component Analysis of metal availability in areas with different Bee Environmental Quality Index levels in Uberlândia, MG.
PCA: Principal Component Analysis; BEQI: Bee Environmental Quality Index.

The widespread presence of metallic contaminants, originating from diverse sources such as vehicular traffic and industrial activities, underscores the complex interactions between urbanization and environmental health. A study using the species *Tillandsia usneoides* (Bromeliaceae), a model species in studies of biomonitoring of air quality, revealed that high concentrations of copper (Cu) and zinc (Zn) were associated with industrial areas and locations near vehicular emissions (Barbosa et al., 2021).

Additionally, other studies have shown that copper is evenly distributed between industrial zones and areas with heavy traffic (Adachi and Tainosho, 2004; Nogueira, 2006; Thorpe and Harrison, 2008). These findings emphasize the need for environmental interventions that go beyond the creation and conservation of green spaces, addressing the reduction of pollution sources in urban areas to effectively protect pollinators and improve the quality of life. Implementing pollutant control measures and strengthening environmental policies are crucial to ensuring that areas classified as having high environmental quality truly function as refuges for bees.

The similarity dendrogram (Figure 3) supports the PCA results, showing that elements such as cadmium (Cd), chromium (Cr), and manganese (Mn) exhibit strong associations, indicating their frequent co-occurrence in urban environments. In contrast, copper (Cu) and calcium (Ca) showed a negative association, possibly reflecting distinct sources for these metals or antagonistic interactions.

The strong correlations among cadmium (Cd), chromium (Cr), and manganese (Mn) suggest that they may originate from common sources, such as proximity to roads and industrial areas. Recent studies have emphasized the role of vehicular traffic and industrial activities in releasing these metals into the environment (Herrero Fernández, 2017; Barbosa et al., 2021; Şeker et al., 2022).

Cadmium (Cd), chromium (Cr), and manganese (Mn) exhibit diverse effects on bees. Manganese (Mn) ingestion alters brain biogenic amine levels, causing bees to initiate foraging prematurely, which reduces their overall number of foraging trips during their lifespan (Søvik et al., 2015). Chronic cadmium exposure significantly decreases the duration, distance, and average speed of flight in *Bombus impatiens* individuals (Gao et al., 2024). Furthermore, cadmium in food resources remains undetectable by *Apis mellifera* bees, as they do not reject sucrose solutions contaminated with Cd, nor does its ingestion alter their sucrose sensitivity (Burden et al., 2019). The combined acute and chronic effects of cadmium and copper on *A. mellifera* impair the fitness of larvae and foragers when these metals are simultaneously present (Di et al., 2020).

Continued research into the relationship between urbanization, metal contamination, and pollinator health is essential. Urban management strategies should focus on both creating and preserving habitats and controlling factors that negatively affect these environments, thereby ensuring the resilience of bee populations and other key organisms essential for urban biodiversity. In this context, pollen collected from melittophilous species like *T. stans* serves as a valuable tool for monitoring metal pollution and providing parameters for assessing environmental quality for bees in urban areas. These findings contribute to advancing knowledge in the field of ecotoxicology, shedding light on how metals interact with and affect urban ecosystems.

Conclusion

This study provides insights into the presence of metal contaminants in the pollen of *T. stans*, a common floral resource for bees in the urban environment of Uberlândia, Brazil. Our analysis confirmed the presence of essential (Ca, Cu, Fe, Mg, Mn) and non-essential/potentially toxic (Al, Cd, Cr) metals in pollen samples collected from areas with varying BEQI classifications.

A key finding was the lack of a statistically significant relationship between the overall metal profiles in pollen and the BEQI classification of the sampling areas. Metal contamination appears to be widespread across the urban landscape, likely driven by diffuse sources such as traffic and atmospheric deposition, exposing bees to metals even in areas considered to have higher environmental quality based on the BEQI. The observed co-occurrence and strong association among Cd, Cr, and Mn suggest common anthropogenic sources and highlight the need to consider the combined toxicological effects of metal mixtures on bee health, which may be more severe than the effects of individual metals.

In conclusion, *T. stans* pollen serves as a potential bioindicator of metal presence in urban environments. Additionally, given the widespread nature of metal contamination, conservation strategies for urban pollinators must go beyond simply providing green spaces and actively focus on mitigating pollution sources.

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Oliveira, C.O.: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing. **Junqueira**, C.N.: formal analysis, methodology, writing – review & editing. **Santos**, D.Q.: formal analysis, methodology. **Assis**, L.C.: formal analysis, methodology, writing – review & editing. **Rocha-Filho**, L.C.: methodology, writing – review & editing. **Nogueira-Ferreira**, F.H.: conceptualization, data curation, formal analysis, investigation, methodology, writing – review & editing. **Augusto**, S.C.: conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing – review & editing.

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