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LETTER

Assessing ecosystem resilience: effects of different environments on native flora, lichens and bee pollen in a mid-sized Brazilian city

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Abstract

The alteration of the natural environment by human activities has increased in recent decades, with a significant impact on the organisms that inhabit it. One of the most apparent consequences of environmental alterations is the occurrence of climatic changes. The city of Joinville is situated in the southern region of Brazil. It has achieved a notable position at the national level, largely due to the significant presence of large-scale industrial operations in the metal-mechanical, plastic, and textile sectors. This study aimed to assess the effects of urban environments on *Nectandra oppositifolia* plants and lichen communities using a passive biomonitoring approach, and on *Tetragonisca angustula* stingless bees using an active biomonitoring approach. This study demonstrated a pronounced effect of anthropogenic activities on plant anatomy and morphology (higher leaf area, fresh mass, dry mass, and water content in the urban-industrial site), with higher metal concentrations in leaf particulate matter ($3.14 \pm 0.4\ \mu g\ cm^{-2}$). The diversity of lichens was the lowest, whereas that of crustose lichens was the highest in industrial-urban and residential-rural sites (industrial-urban site = 13 species, residential-rural site = 29 species, control site = 33 species, $p < 0.05$). The distribution of species within the lichen communities was inversely correlated with the degree of anthropogenic influence. Some lichen species were found exclusively in the control areas, suggesting that they may indicate good environmental quality (*Arthonia sp., Astrothelium sp., Phyllopsora pyxinoides, Phyllopsis sp., and others*). Beehive pollen analysis revealed the presence of Zn, Cr, Ni, Cu, and Pb, and industrial-urban site exhibited higher metal concentrations (particularly, Ni, 12.59 ± 0.9, and Cr, 3.76 ± 1.00, mg kg^{-1}). This approach highlights the environmental repercussions that affect the Plantae, Fungi, and Animalia kingdoms. The use of biomonitoring as a robust tool in good environmental quality assessment provides insight into policy decisions and underscores the importance of pollution studies.

1. Introduction

The acceleration of industrialization, coupled with a large increase in the number of vehicles, has contributed significantly to the deterioration of the environment in urban areas, introducing a variety of pollutants and contributing to areas generally classified as having poor environmental quality. This phenomenon represents a

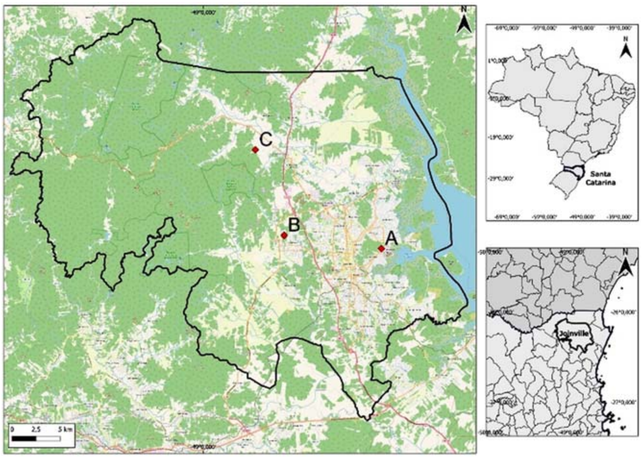

Figure 1. Location of Joinville (SC, Brazil) and sampling areas selected for study.

significant threat to the global society, negatively impacting human health, ecosystems, and the economy. Despite the abundance of research on urban enviromental quality in megacities, there is a lack of understanding of its implications in small-to medium-sized cities [1, 2]. Joinville, a mid-sized city situated in the northeast region of Santa Catarina, southern Brazil, serves as a prominent industrial center with a population of 616,323 inhabitants and over 2,000 industries, mainly in the metal-mechanic, plastics, and textile sectors [3, 4].

Monitoring of environmental quality through physical and chemical methods has become increasingly prevalent. However, these approaches often fail to provide immediate conclusions regarding the impact of environmental pollutant concentrations on living beings. Consequently, there is growing emphasis on the use of bioindicators and biomonitoring techniques to assess environmental quality in an efficient and cost-effective manner [5, 6]. Several studies have demonstrated the effects of pollutants on various organisms, including plants, lichens, and insects, using biomonitoring methods [7–9].

Plants exposed to environments in the presence of different pollutants, in any environmental compartment (soil, water and air), exhibit a range of alterations in vascular bundle area, phloem cell collapse, alterations in trichome density and disturbances in photosynthesis [5, 10, 11]. Lichens are particularly susceptible to pollutants because of their distinctive biology, which makes them highly valuable bioindicators in biomonitoring programs. Nevertheless, research on the environmental effects of lichen communities in Brazil is limited to metropolitan regions [12–14]. Furthermore, bees and their products also act as indicators of environmental contamination, as they can accumulate pollutants from the air and flora during foraging [8, 15, 16]. Stingless bees, native to Brazil, offer particular advantages in biomonitoring because of their widespread distribution, habitat versatility, and ease of colony manipulation [17, 18].

The objective of this study was to assess the response of diverse biological systems, including plants, lichens, and bees, to environmental quality in both urban and rural settings.

2. Material and methods

2.1. Study area

Joinville is situated in the north-eastern state of Santa Catarina, it is coastal city in southern Brazil (figure 1). It covers an area of 1,134.03 km^2 and is located within the Atlantic Forest biome. This biome is characterized by a variety of forest formations, including dense lowland rainforests, mangroves, submontane dense rainforests, montane dense rainforests, and high-montane dense rainforests. Due to regional topography and coastal position, Joinville is characterized by high humidity and prevailing wind directions are usually from East. The climate of Joinville is classified as Cfa according to Köppen's classification, which indicates a 'mesothermal, humid climate without a dry season.' The average annual humidity is 76.04%, and the temperature is 22.63 °C. The city is the largest in Santa Catarina State, with an estimated population of 616,323, serves as a prominent industrial center, particularly in the metal-mechanical, plastic, and textile sectors. This is the third economic pole of southern Brazil [4].

In this study, three sampling areas were selected with an average distance of 15 km between them, allowing for the differentiation of each sampling point in terms of its characterization of the local area. Furthermore, consideration was given to the prevailing wind directions and the geographical features since the dispersion of pollutants is directly influenced by atmospheric characteristics, topography and emission sources.

Figure 2. Geographical distribution of industrial companies in the city, (a) small- (b) medium- (c) large.

The occurrence of winds from the east (26.5%) and northeast (16.4%) is more frequent. During the summer, winds from the east and northeast are predominant, while during the winter, winds from the southeast and south are more prevalent. The average wind speed is 10 km h^{-1}. Joinville is situated between Serra do Mar mountain (to the west), which is covered by the remains of the Atlantic Rainforest, and the estuary of Babitonga Bay (to the east). By consulting the Joinville council website, it was possible to obtain the following information for each company: name, industrial size, sector, and location. This data was then processed using the Microsoft Power BI and Power Query software to create a satellite image of the city, including the geographical distribution of the industrial companies in the city (figure 2), classified according to their size. The sampling area A is defined as a representative urban-industrial site, characterized by high traffic volumes, including buses, trucks, and personal vehicles, in addition to a prevalence of large, medium, and small-scale industrial operations. These include a significant Metallurgical Industrial Complex. Additionally, the area exhibits a significant concentration of commercial and residential activities. The sampling area B is characterized as residential-rural site, and distinguished from sampling area A by a concentration of moderate and smaller-scale industrial operations, predominantly within the plastic manufacturing sector. In addition, the area shows a moderate level of commercial activity, with agricultural land (mainly rice fields) dominating. The sampling area C was located in the vicinity of a forest fragment and is characterized by a low level of exposure to potential sources of contamination.

2.2. Botanical material collection and analysis

The species Nectandra oppositifolia has been observed across all sampling areas. It is commonly referred to as 'rust cinnamon' or 'yellow cinnamon,' and is native to various Brazilian biomes. It is a large tree, reaching up to 30 meters in height. Fifteen adult trees (five from each sampling area) were selected for morphological and anatomical analyses. The collections were conducted during the same phenological phase of the tree and in the same season of the year, with the objective of standardizing the data and neutralizing the effect of seasonality on the populations. From each adult tree, 35 fully expanded leaves of the outer canopy were collected, fixed at the third node, and free of herbivorous lesions (25 for morphological and 10 for anatomical analyses), resulting in a total of 175 leaves per sampling point. The following morphological attributes were considered: leaf mass, area, density, and water content. Histological preparations and optical microscopy were employed to analyze anatomical characteristics, including epidermal thickness and parenchymal dimensions [19]. The leaf particulate matter concentration involved the collection of 25 leaves from each of the 15 adult trees, resulting in 125 samples per area. The leaf particulate matter (PM) mass was deduced by cleaning the leaf surfaces to remove the deposited matter, followed by quantification of the leaf area (cm^2) using Sigma Scan Pro 5.0 software. PM concentration was calculated by dividing the PM mass by leaf area ($\mu g\ cm^{-2}$). Metal concentrations in PM were evaluated by acid extraction using concentrated double-distilled nitric acid followed by inductively coupled plasma optical emission spectrometry (ICP-OES) technique for analysis of Pb, Cu, Cr, Ni, and Zn. In order to guarantee the quality of the results obtained, high purity grade chemical reagents were used, as well as certified standard reference solutions.

2.3. Lichen collection and analysis

Nectandra oppositifolia (Lauraceae) was selected as the phorophyte because of its prevalence across all sampling areas and its favorable lichen colonization potential. Lichens were collected from 15 phorophytes, with five adult individuals per area, for a total of 45 phorophytes, covering both sunny and shady sections. Trees were chosen from open spaces rather than from forests. The lichen community was characterized using the elastic method [20], with the measured area of the phorophyte ranging from 30 cm to 180 cm in height and 15 cm intervals, as well as correlated with environmental factors, adjusting the constant K to 11. Specimens were collected, preserved, and identified using standard lichenological methods [21]. Morphotypes lacking sexual reproduction structures have been noted, awaiting molecular studies for definitive taxonomy. Differentiation was based on the texture, color, shape, and propagule layout. The characterization and identification of the specimens involved

Table 1. Biological attributes of Nectandra oppositifolia (Lauraceae) in urban- industrial (A and B) and residential-rural (C) areas of Joinville/SC, Brazil.

Attribute	Sample area A	Sample area B	Sample area C
Specific leaf area (SLA, $cm^2 g^{-1}$)	68.61 ± 32.7	72.3 ± 56.53	67.83 ± 38.08
Leaf area (LA, $cm^2 g^{-1}$)	54.38 ± 22.81	36.4 ± 15.56	30.53 ± 15.8
Water content (WC, g cm^{-2})	0.7 ± 0.35	0.57 ± 0.27	0.51 ± 0.36
Leaf density (LD, $mm^3 g^{-1}$)	> 0.01	> 0.01	> 0.01
Abaxial cuticle thickness (ABCT, μm)	2.6 ± 0.8	2.03 ± 0.64	2.23 ± 0.61
Adaxial cuticle thickness (ADCT, μm)	4.7 ± 1.22	3.18 ± 0.92	2.35 ± 0.47
Leaf thickness (LT, μm)	217.05 ± 28.86	230.99 ± 28.79	211.78 ± 21.87
Mesophyll thickness (MT, μm)	209.75 ± 28.48	225.78 ± 28.58	207.2 ± 21.75
Fresh mass (FM, g)	1.57 ± 0.69	1.2 ± 0.54	1.03 ± 0.6
Dry mass (DM, g)	0.87 ± 0.41	0.63 ± 0.29	0.52 ± 0.29
Lacunous parenchyma (LP, μm)	66.82 ± 14.39	66.68 ± 8.25	57.42 ± 8.97
Palisade parenchyma (PP, μm)	95.13 ± 15.03	101.61 ± 14.91	101.98 ± 10.88
Leaf particulate matter (LPM, μg cm^{-2})	3.14 ± 0.4	0.13 ± 0	0.15 ± 0

the use of stereoscopic and optical microscopy as well as the identification of secondary metabolites [22, 23]. The collected samples were deposited at the JOI Herbarium, University of the Joinville Region, UNIVILLE.

2.4. Pollen collection and metal analysis

The study was conducted using colonies of the stingless bee species Tetragonisca angustula (Latreille, 1811) (jataí), with one colony in each sampling area. The hives were installed in rational boxes on stands with the purpose of preventing the invasion of ants and other insects. The height of the boxes was 1 m, and they were covered with tiles for thermal and rain protection. From each nest, pollen samples were collected periodically, every two months for one year. The pollen was taken directly from the food pots, placed in sterile plastic-capped jars, labeled, and stored under refrigeration at 5 °C. Metal concentrations were quantified by weigthin approximately 1 g of ground and sieved pollen into test tubes, 10 ml of nitric acid PA was added, and the mixture was heated until complete digestion of the samples. Then the samples were filtered and transferred to 25 ml volumetric flasks and the final volume was completed with ultrapure water (Milli-Q). Metal concentrations (Pb, Cu, Cr, Ni, and Zn) were evaluated by the inductively coupled plasma optical emission spectrometry (ICP-OES) technique [7]. In order to guarantee the quality of the results obtained, high purity grade chemical reagents were used, as well as certified standard reference solutions.

2.5. Statistical analysis

To compare the means, multivariate analysis (ANOVA) and the Tukey test with a p-value of less than 0.05 were employed. Principal component analysis (PCA) was used to explain variations in biological and environmental variables [24]. Similarities between lichen species composition from different areas were estimated using a Sorensen coefficient. (QS). Pearson's correlation was used to show possible trends between lichen diversity and environmental pollutants. All analyses were performed in the R statistical environment [25].

3. Results and discussion

3.1. Biological attributes of Nectandra oppositifolia species

According to the literature, plants respond to different environmental pollutants in different environmental compartments (air, soil, and water), manifesting symptoms such as shading, stomatal closure, and reduced CO_2 flow [11, 26]. Exposure to atmospheric pollution is one of the main causes of cell disorganization, as evidenced by plant structures [5]. Pollutants may induce physiological and biochemical changes that inhibit enzymatic and photosynthetic activities, alter cuticle and wax production, and cause necrosis, chlorosis, and leaf fall [11, 26]. In this study, significant variations in the biological attributes of *Nectandra oppositifolia* were observed. The species exhibited a higher leaf area, fresh mass, dry mass, and water content in sampling areas A (urban-industrial site) and B (residential-rural site) than in the control site (table 1).

In sampling area A, which was in close proximity to industrial and traffic-related activities, a higher concentration of foliar PM was observed, which was 30 times higher than that observed in areas B and C (table 1). Some authors have observed significantly higher leaf particulate matter concentrations in urban areas than in rural areas, which can hinder photoreceptor radiation capture, plant respiration, and transpiration. In addition, a reduction in chlorophyll levels can be observed due to the accumulation of particulate matter in the leaves [26, 27]. This suggests that particles adhere to the epicuticular wax, causing shading effects and negative

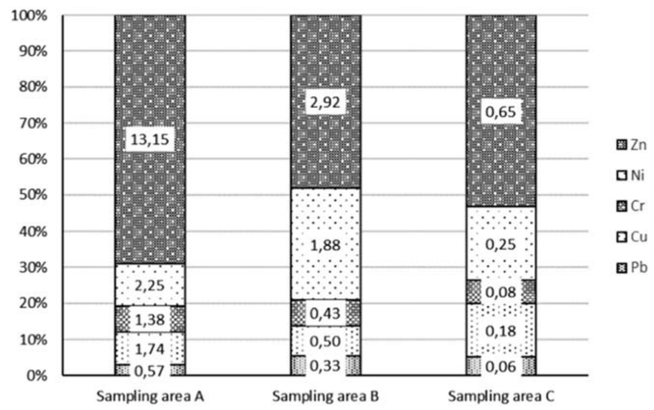

Figure 3. Distribution of metal concentrations (mg g^{-1}) in leaf PM.

impacts on plants, even after washing. Despite this, *Nectandra oppositifolia* showed an increase in leaf area, which may have been a compensatory response to biochemical material loss, suggesting a plasticity characteristic of this species when living in urban environments. Palisade parenchyma thickness was the lowest in urban areas, while the lacunar parenchyma thickened, possibly to compensate. Leaf thickness did not show any significant differences. In addition, the adaxial cuticles exhibited thickening, probably in response to varying leaf particulate matter concentrations, in contrast to the abaxial cuticles. Differences in particulate matter accumulation between adaxial and abaxial leaf surfaces have been observed, with adaxial surfaces collecting more particles [28]. This suggests that *N. oppositifolia* adapts to urban conditions by adjusting leaf morphology to meet the increased nutritional demands. Soil conditions were not analyzed in this study, but plant response is also strongly influenced by the quality of this environmental compartment [26–28]. It would be possible to suppose that, as assumed for air quality, in terms of foliar particulate matter concentrations, and metal concentrations (results presented below), better conditions for water and soil quality would be expected in the control sampling area, reinforcing the results observed in table 1.

Metal concentrations were quantified in *Nectandra oppositifolia* leaf particulate matter. The results indicate that Zn exhibited the highest concentration, followed by Ni, Cu, Cr, and Pb. The concentration of Zn in the urban-industrial site was 4.5 times higher than in the residential-rural site and 20 times higher than in the control sampling site (figure 3). The concentration of metals in leaf particulate matter collected in the control sampling area was notably lower than that observed in the industrial-urban site. In particular, the levels of Zn and Cu were approximately 20 times lower, while the concentrations of Ni, Cr, and Pb were around 10 times lower in the control sampling area. The concentration of metals in the industrial-urban site was also found to be higher than in the residential-rural site, with a range of 2 to 4.5 times higher in the industrial-urban site than in the residential-rural. A major metallurgical industrial complex is located in close proximity to sampling area A. Industrial activities of this nature contribute to environmental degradation, particularly by the release of metals (As, Pb, Cr, Ni, Cd, Mn, Fe, Zn, and Cu) into the environment. It is possible that this industrial activity is influencing the metal concentrations observed in the leaf PM collected in this area. Additionally, the dispersion of pollutants is not optimal due to low wind velocity. Furthermore, it is possible that this industrial activity is influencing the metal concentration in other environmental compartments (water and soil), which could subsequently affect the development of the *Nectandra oppositifolia* plant, as previously discussed. These findings are consistent with the results observed for bee pollen (see results presented later in the text). Furthermore, consideration was given to the prevailing wind directions and the geographical features since the dispersion of pollutants is directly influenced by atmospheric characteristics, topography and emission sources.

Principal component analysis (PCA) of leaf attributes was conducted, and according to figure 4, the first two axes of the PCA explained 60.1% of the variation in the dataset. Principal component 1 contributed to 42.53% of this variation, represented by the attributes of fresh mass, dry mass, water content, and leaf area. Principal component 2 accounted for 17.57% of the variation and represented the thickness attributes of the abaxial and adaxial faces of the epidermis and palisade parenchyma.

Plants play a significant role in the monitoring of environmental quality, for example, mitigation of the adverse health effects of air pollution, and management of urban green spaces [10, 29]. They can filter particulate matter from the air, with variations according to plant species, accumulating on their leaf surfaces and waxes [30]. Leaf blades are pivotal in capturing atmospheric particulate matter, with differences in surface characteristics influencing retention abilities. Generally, tree species with thicker epicuticular wax layers capture particulate matter more effectively than broad-leaved species. Additionally, leaves with more pubescence and

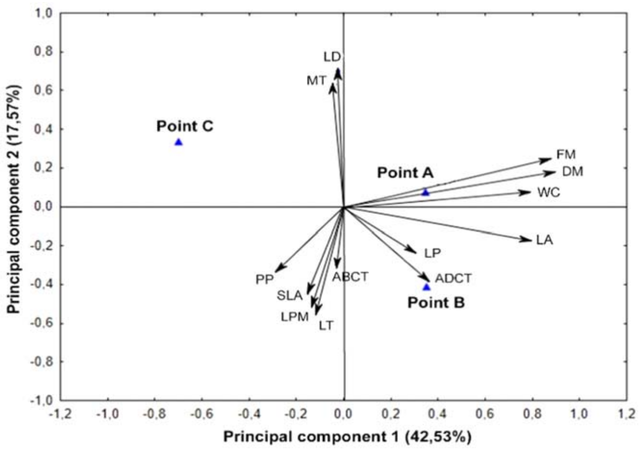

Figure 4. Principal component analysis of leaf attributes of *Nectandra oppositifolia* (Lauraceae) occurring in the sampling areas of Joinville, Santa Catarina.

Table 2. Species richness, absolute dominance (DoA) values, and percentages of lichen community occurring at the sampling areas.

			(%)		
Sampling area	Richness (number of species)	DoA	Crustose	Foliose	Squamulose
A	13	0.107	92.85	7.15	0
B	29	0.164	75.86	10.34	13.8
C	33	0.365	81.81	12.12	6.06

rougher surfaces tend to accumulate greater amounts of particulate matter. Trees in urban and industrial areas play a crucial role in the reduction of airborne particulate matter. At the same time, this pollution negatively affects the health of trees, necessitating consideration in urban forest management. The amount of particulate matter on leaves depends on leaf quantity, size, and morphology and is influenced by epicuticular wax. There was a tendency for higher wax production in the studied species when growing under particulate matter pollution stress. Additionally, pollution reduces photosynthetic efficiency and impairs normal plant functions [29].

3.2. Diversity and structure of the lichen community

Regarding lichen species, 47 species were identified, distributed in 27 genera and 16 families, of which 46 species are crustose (microlichens), seven foliose species, and four squamulose species (macrolichens). *Arthoniaceae* is the best-represented family with six genera and 11 species, followed by the *Graphidaceae* family with 3 genera and 6 species. The remaining specimens corresponded to the families *Trypetheliaceae* with three genera and four species, *Porinaceae* with one genus and four species, *Ramalinaceae* with three genera and six species, *Coegoniaceae* with one genus and three species, *Verrucariaceae* with two genera and two species, *Opegraphaceae* and *Physciaceae* with one genus and two species each. The families *Arthopyreniaceae*, *Collemataceae*, *Monoblastiaceae*, *Pannariaceae*, *Parmeliaceae*, *Pilocarpaceae*, and *Stereocaulaceae* contained only one species each.

As shown in table 2, industrial-urban site exhibited the lowest diversity, with 13 species, while residential-rural site demonstrated 29 species, and control sampling area exhibited the highest diversity, with 33 species, indicating a significant difference among the sample areas ($p < 0.05$). The lichen species increased with distance from heavily industrialized and urbanized area, suggesting that the region is becoming inhospitable for many species. Lichens are valuable for biomonitoring the quality of environment because of their sensitivity to pollutants, and many researchers have observed a lower diversity of urban lichen communities with a corresponding decrease in richness near pollution sources [9, 13, 14, 26, 28]. It is also important to note that the ability of lichens to grow is closely related to their ecophysiological characteristics and is dependent on fluctuations in light intensity, temperature, and humidity. In general, there is a decrease in relative humidity and an increase in temperature and light incidence away from the edge [20, 27].

The industrial-urban site exhibited the lowest absolute dominance value (0.107), whereas control sampling site showed the highest (0.365). Industrial-urban site had the highest percentage of crustose lichens (92.85%), whereas control sampling site and residential-rural site had higher percentages of foliose species (12.12% and 10.34%, respectively) (table 2). Foliose species are more sensitive to environmental changes, which can result in an increase in crustose species when foliose species disappear [27]. This trend is evident in industrial-urban site, which is heavily affected by urban and industrial activities, where pollution-tolerant species are dominant, suggesting a substitution in lichen communities among sample areas. The lichens *Porina distans* and *Herpothallon rubrocinctum* exhibited the highest frequencies across all areas, indicating their resilience to environmental pollution. The species with the highest Importance Value (VI) were *Porina distans* (74.84%) in industrial-urban site, CBE - sterile white crust lichen (42.14%) in residential-rural site, and CVS - sorediate green crust lichen (56.69%) in control sampling site.

A number of species were observed exclusively in the control sampling site, including *Arthonia* sp., *Astrothelium* sp., *Phyllopsora pyxinoides*, *Phyllopsis* sp., *Cryptotecia striata*, *Diorygma hieroglyphicum*, *Flakea papillata*, *Graphis* gr. *subserpentina*, *Lecanactis* aff. *totarae*, *Opegrapha ochroplaca*, *Parmotrema lobulatum*, *Physcia sorediosa*, *Phaeographis* sp., *Syncesia rhizomorpha*. These species may serve as indicators of good environtmental quality, warranting further attention in future research to enhance the accessibility of such information. In a study conducted in Porto Alegre, southern Brazil [12], *Anisomeridium tamarindii* (Fée) R. C. Harris and *Pertusaria carneola* (Eschw.) Müll. Arg. were identified as indicator species for environmental quality. *Canoparmelia* sp., *Dirinaria picta*, *Heterodermia obscurata*, *Parmotrema tinctorum*, *Physcia aipolia*, *Punctelia graminicola*, *Teloschistes exilis*, and *Usnea* sp. have been classified as characteristic species of altered environments in studies conducted in Porto Alegre, Brazil [27]. Canoparmelia texana has been identified as a pollution-tolerant species [31] and has been utilized in air pollution studies in São Paulo [20].

Sample areas B and C (residential-rural site and control sampling site) showed similar species composition (Sorensen Index = 0.322). In contrast, the industrial-urban sampling site showed the lowest degree of similarity (0.086), suggesting the presence of high levels of pollutants in one or all environmental compartments (soil, water and air), which has a detrimental effect on lichen diversity. The control sampling site showed high species richness, including indicator species for good environmental quality, such as foliose lichens and squamulose lichens.

By correlating the variables lichen cover and metal concentrations analyzed in leaf particulate matter, it was observed that a decrease in lichen cover is associated with elevated levels of Zn, Ni and Cu ($r = -0.37$, $p < 0.05$, $r = -0.25$, $p < 0.05$, $r = -0.34$, $p < 0.05$, respectively).

3.3. Metals concentration in pollen from stingless beehives

In addition to serving as indicators of environmental quality, bees, pollen, and honey, are bioindicators that reflect the presence of pollutants in the environment [7, 15, 18]. Pollen samples are frequently employed in air pollution studies [7, 8, 32]. Pollutants are carried into bee colonies by bees, which collect nectar and pollen and accumulate in bee products, reflecting environmental conditions [16], as well as elevated stress and neurotoxic pollutant levels present in bees from regions with greater human influence [18]. The city of Joinville is the largest in Santa Catarina State, with an estimated population of 616,323, serves as a prominent industrial center, particularly in the metal-mechanical, plastic, and textile sectors. This is the third economic pole of southern Brazil and contains of 19 of the 500 biggest industries of Brazil [4].

According to the elements quantified in this study, although Zn, Ni, and Cu are essential for living organisms, concentrations above a certain threshold may be toxic. The metals Cr and Pb are non-essential elements that are highly toxic to organisms and are commonly used in studies on environmental pollution because their presence is associated with industrial activities and the excessive use of pesticides in rural areas. In this study, the highest concentrations were observed for Zn, followed by Ni, Cu, Cr, and Pb (figure 5). Except for Ni and Cr, all metals were found in higher concentrations in sampling areas A and B than in sampling area C (control sampling area). The concentrations of Zn, Cu, and Pb in the control sampling area were approximately half those observed in sampling areas A and B, whereas the concentrations of Ni and Cr were similar. This similarity demonstrates the extensive range of potential sources of pollutant emissions, which are influenced by the speed and direction of the wind, as well as the topography of the region. In the study region, winds prevail from the east (26.5%) and northeast (16.4%) for the majority of the year, with a speed of 10 km h^{-1}. The sampling control area is situated between the Serra do Mar mountain (to the west) and the estuary of Babitonga Bay (to the east), and potential pollutants could reach this region and accumulate there. It is notable due to the presence of all metals analyzed in the control sampling area. The sampling areas A and B exhibit a greater anthropogenic impact due to commercial and industrial activities than control sampling area. Other studies [7, 8, 32] also reported higher metal concentrations in pollen samples from urban-industrial and residential-rural sites than in those from control sites. In a study conducted in Jordan, the concentrations of Pb, Cu, Zn, and

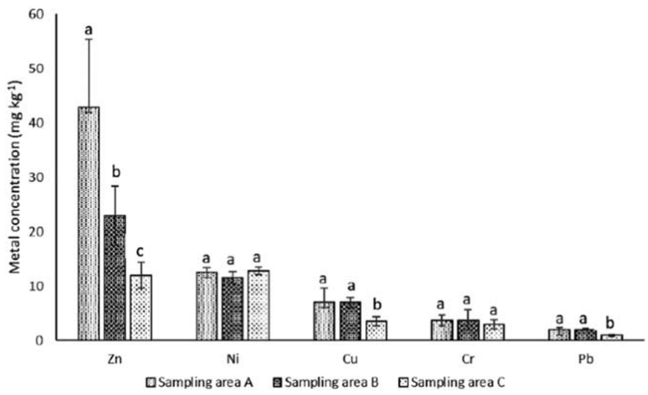

Figure 5. Metal concentration in bee pollen.

Ni in pollen samples ranged from 0.03 to 2.57 mg kg^{-1}, 0.032 to 11.39 mg kg^{-1}, 25.24 to 77.02 mg kg^{-1}, and 0.01 to 2.81 mg kg^{-1}, respectively. The samples were drawn from rural, deserted, and industrial areas. The mean value of Pb in agricultural areas was 0.326, in industrial areas 0.358, and in desert areas 0.020. In a study of the mineral elements and heavy metals present in bee pollen manufactured in different regions of Turkey [7], a range of metal concentrations was observed (minimum-maximum, mg/kg pollen): Cr 0.124–1.595, Cu 3.728–14.994, Fe 28.603–725.360, Mg 271.107–1278.340, Mn 8.151–201.036, Se 0.593–5.085, Zn 14.832–39.079, B 0.592–61.33, Ni 0.002–1.761, Si 0.082–10.500, K 992.107–2894.154, Ca 491.853–1472.102, P 795.899–5246.992, Cd 0.006–0.181, Pb 0.000–0.479, and As 0.006–1.035. The authors conclude that the bee pollens consumed in Turkey may serve as a valuable source of minerals, while the concentration of heavy metals (Cd, Pb, and As) are below the limit maximum established by the international legislation. In comparing of these results with those obtained in this study is important to highlight the elevated concentrations of Ni and Cr, which were approximately 10 and 2 times, respectively, higher than the maximum concentrations observed in the aforementioned studies. Ni is one of the metals more used by the metallurgical industries, as well as the metal Cr, both are also used for the surface treatment of the products.

Equal letters in a group (sampling areas) do not differ statistically at the 5% significance level, according Tukey´s test ($p \leqslant 0.05$), $n = 6$.

The concentrations of inorganic contaminants in bee honey are regulated by the Codex Alimentarius and Brazilian laws, which set maximum levels based on toxicity and cumulative tolerance in humans [33, 34]. While there are no established limits for inorganic contaminants in pollen, Brazilian regulations specify maximum levels for Pb (0.3 mg kg^{-1}), Zn (50.0 mg kg^{-1}), Ni (5.0 mg kg^{-1}), and Cr (0.1 mg kg^{-1}) in food [34]. According to Codex, the maximum permissible levels of inorganic contaminants in honey are 1 mg kg^{-1}, 2 mg kg^{-1}, and 2 mg kg^{-1} for arsenic, lead, and copper, respectively. These levels are notably lower than those observed in this study, particularly in the pollen samples obtained from bee colonies located in the industrial-urban site, where higher concentrations are typically found. Hives located in industrial-urban and residential-rural sites produced bee products with a high concentration of pollutants, and the presence of metals in pollen was the evidence.

4. Conclusion

The results indicated a lower richness and diversity of lichens in industrial-urban and residential-rural sites than in control site. Additionally, some lichen species were found to be indicative of high or low anthropogenic influence. The structural characteristics of plant species in industrial-urban and residential-rural sites were also affected. Pollen produced in industrial-urban and residential-rural sites, which can be consumed by humans, has higher concentrations of metals. The proxy approach for measuring environmental quality, which employs response variables associated with different groups of organisms, represents a valuable tool that can be utilized in a multitude of regions worldwide. This enhances the reliability of the results and conclusions regarding environmental pollution and may assist in the formulation of policies for monitoring indusrial-urban and residential-rural sites. Given the unique characteristics of these sites, a more meticulous consideration of the environmental quality parameters is necessary. This underscores the importance of biomonitoring as a pivotal tool in this context.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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