Ecotoxicology and Environmental Safety 215 (2021) 112147

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of native forest and human-modified land covers on the accumulation of toxic metals and metalloids in the tropical bee *Tetragonisca angustula*

Marcela de Matos Barbosa [a], Ana Carolina Coelho Fernandes [b], Rafael Souza Cruz Alves [c], Denise Araujo Alves [d], Fernando Barbosa Junior [b], Bruno Lemos Batista [e], Milton Cezar Ribeiro [c], Maria Fernanda Hornos Carneiro [b,f,*,1]

[a] *Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil*
[b] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil*
[c] *Laboratório de Ecologia Espacial e Conservação, Departamento de Ecologia, Universidade Estadual "Julio de Mesquita Filho", Rio Claro, Brazil*
[d] *Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil*
[e] *Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, Brazil*
[f] *Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile*

ARTICLE INFO

Edited by: Paul Sibley

Keywords:
Biomonitoring
Environmental pollution
Landscape heterogeneity
Ecotoxicology
Stingless bees
Tetragonisca angustula

ABSTRACT

The intensive shift on land cover by anthropogenic activities have led to changes in natural habitats and environmental contamination, which can ultimately impact and threat biodiversity and ecosystem services, such as pollination. The aim of this study was to evaluate the effect of native forest and human-modified land covers on the concentrations of chemical elements accumulated in the neotropical pollinator bee *T. angustula*. Eight landscapes, within an Ecological Corridor in the State of São Paulo, Brazil, with gradients of forest cover, spatial heterogeneity and varying land covers were used as sampling unities. Bees collected in traps or through actives searches had the concentration of 21 chemical elements determined by ICP-MS. Results show a beneficial effect of forested areas on the concentrations of some well-known toxic elements accumulated in bees, such as Hg, Cd, and Cr. Multivariate Redundancy Analysis (RDA) suggests road as the most important driver for the levels of Cr, Hg, Sb, Al, U, As, Pb and Pt and bare soil, pasture and urban areas as the landscape covers responsible for the concentrations of Zn, Cd, Mn, Mg, Ba and Sr in bees. The results reinforce the potential use of *T. angustula* bees as bioindicators of environmental quality and also show that these organisms are being directly affected by human land use, offering potential risks for the Neotropical ecosystem. Our study sheds light on how land covers (native forest and human-modified) can influence the levels of contaminants in insects within human-dominated landscapes. The generation of predictions of the levels of toxic metals and metalloids based on land use can both contribute to friendly farming planning as well as to support public policy development on the surrounding of protected areas and biodiversity conservation hotspots.

1. Introduction

Land cover changes, such as the conversion of natural habitats to agriculture, industrial activities and urban expansion, have led to environmental contamination and changes in the natural habitats, thus impacting biodiversity and ecosystem functions and services (Ali et al., 2019; Azad et al., 2019; Chara-Serna et al., 2019; Ponte et al., 2019; Tenkouano et al., 2019). Because of that, risk assessment of pollutants, such as toxic metals, has been the subject of ecotoxicological discussions in recent years (Sodango et al., 2018; Urrutia-Goyes et al., 2018). Toxic metals such as mercury (Hg), lead (Pb) and cadmium (Cd) as well as the semimetal arsenic (As) are not degradable and there is no homeostatic role exerted by them. Although they are "natural" contaminants, the input of these chemical elements into the environment is usually the

* Correspondence to: Facultad de Química y de Farmacia, Campus San Joaquín, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna, 4860 Macul, Santiago, Chile.

E-mail address: maria.hornos@uc.cl (M.F. Hornos Carneiro).

[1] ORCID: 0000-0001-7041-6923

https://doi.org/10.1016/j.ecoenv.2021.112147
Received 9 June 2020; Received in revised form 7 March 2021; Accepted 10 March 2021
Available online 20 March 2021

result of anthropogenic activities (Tenkouano et al., 2019) and depending on the levels they are presented, they may potentially threat several forms of life (Tong and Lam, 2000). Such activities include vehicular emissions (e.g. trace elements adhered to particulate matter from vehicle exhaust, worn tires, worn road surfaces, wear of brake lining, etc.), industrial emissions (e.g. power plants, coal combustion, metallurgical processes) and weathering of the construction and surface of the pavement, as well as mining, waste/sewage disposal and the use of pesticides and fertilizers (Sezgin et al., 2004; Li et al., 2008; Luo et al., 2009; Ibe et al., 2018; Khosheghbal and Esmaeilzadeh, 2018). Many studies show that toxic metals and semimetals can accumulate in the tissues of different wildlife animals, thus affecting nervous, reproductive and immune systems in addition to produce genotoxic damage (Tchounwou et al., 2012; Gizaw et al., 2020).

Bioindicators can be defined as species, communities or biological processes that give early and sensitive responses depending on the environment quality (Holt and Miller, 2011). For example, lichens are good bioindicators of environmental quality because they can manifest inhibition of growth and development of the stalk, anatomical and morphophysiological changes under contaminated conditions. This occurs because of the high sensitivity of lichens (cell membrane damage in response) to several pollutants (Osyczka and Rola, 2019). Another example of bioindicator is the *Bauhinia blakeana tree*, because, when exposed to air pollution, it presents higher pollen abortion rates, a response directly related to a drop in environmental quality (Carneiro et al., 2011). In addition to lichens and vascular plants (Sumita et al., 2003; Grembecka and Szefer, 2013; Kłos et al., 2018; Huihui et al., 2020; Zhang et al., 2020), bees (Porrini et al., 2003; van der Steen et al., 2012; Silici et al., 2016; Giglio et al., 2017; Zarić et al., 2017, 2018) and its products such as honey (Stecka et al., 2014; Bilandžić et al., 2017), pollen, propolis and wax (Formicki et al., 2013; Tlak Gajger et al., 2016) have also been used in environmental risk assessments through the evaluation of the bioaccumulated levels of metals and metalloids. For instance, honeybee products collected in areas of intensive vehicle traffic and industrial and agricultural activities presented high concentrations of toxic metals and metalloids in Bulgaria and China (Zhelyazkova, 2012; Zhou et al., 2018). Bees can provide useful information regarding environment quality, especially due to their foraging behavior and hairy bodies, presenting great relevance for monitoring the impacts of anthropogenic land use (Eeva et al., 2004; Corbi et al., 2011; Mihaly Cozmuta et al., 2012; Azam et al., 2015; Skaldina et al., 2018). In Brazil, the widespread exotic honeybee *Apis mellifera* has already been described as a bioindicator of environmental quality on different landscape contexts (Orsi et al., 2006; Morgano et al., 2010; de Oliveira et al., 2016). However, current knowledge is still scarce regarding biomonitoring of native social bees, which present an intimate relation with the environment and have important values to ecosystem health and economies.

Along the Cantareira-Mantiqueira Ecological Corridor, State of São Paulo, Brazil, the native stingless bees *Tetragonisca angustula* play a fundamental role in agroecosystems, particularly because it is a pollinator of several crops (Malagodi-Braga and Kleinert, 2004; Slaa, 2006; Vossler, 2018). Similarly, to what was also observed for *Apis mellifera* honeybees, stingless bees such as *T. angustula*, so-called jataí, live in permanent colonies consisting of a single queen and several workers, who collect pollen and nectar to feed larvae within the colony (Fletcher et al., 2020). Although extremely vital to the ecosystem maintenance, these stingless bees have been exposed to several threats such as forest loss and fragmentation per se (Fahrig, 2003), which results into different anthropogenic land covers across the space. As a consequence, human-modified landscapes are frequently submitted to several environmental pollution sources.

Atlantic Forest, a biodiversity hotspot (Myers et al., 2000), has become increasingly fragmented and heterogeneous due to the conversion of forest cover into pasture, urban and agricultural areas (Tabarelli et al., 2010). It is estimated that forest cover is about 16% of the original area with the small and isolated forest remnants under severe edge effects (Ribeiro et al., 2009). Such changes in the landscape may result in loss of the diversity of species essential for maintaining the ecosystem, such as stingless bees (Cane, 2001; Steffan-Dewenter et al., 2002). Despite their significant environmental importance, a decline in the population of these pollinators has been observed and may be related to the intense modifications on land cover and forest loss, since some of these insects rely on trees to build their nests and establish their colonies (Biesmeijer et al., 2006; Potts et al., 2010; Cameron et al., 2011).

The number of studies dedicated to understand the main drivers of pollinators and insect threats is rapidly increasing worldwide, particularly in Neotropics (Martello et al., 2016; Boscolo et al., 2017; Medeiros et al., 2019). These areas have been continuingly changed with respect to their natural habitat cover, and therefore, many insects can be exposed to levels of contaminants that produce toxicity within human-dominated landscapes. One example is the building of roads and highways. It is known that vegetation barriers aside roads and highways alter the dispersion of vehicular traffic emissions which frequently contain significant levels of toxic metalloids and metals (Nowak et al., 2013b; Cavanagh et al., 2009) which in turn can later bioaccumulate and affect living organisms (Agnan et al., 2015; Zarić et al., 2018; Costa et al., 2019). Also, there is evidence that vegetation filters gases, particulate matter and toxic metals adsorbed on the latter, what contributes to improve air quality (De Nicola et al., 2008; Manisha and Suresh Pandian, 2016; Esposito et al., 2019). Moreover, there is evidence that human land uses had a stronger influence on toxic metals and metalloids concentrations within modified landscapes, either in air, soil, water or in biological entities (Fritsch et al., 2011; Li et al., 2015; Liu et al., 2016; Kodnik et al., 2017; Richardson and Moore, 2020). However, most of the previous studies have focused on ecological and toxicological approaches separately, which stresses the need for further development of multi-scale ecotoxicological studies, thus resulting in the advance of our understanding of pollutant dynamics and toxic effects in ecosystems. In this context, the main goal of this study was to evaluate the effect of native forest and human-modified land covers on the concentrations of chemical elements accumulated in the neotropical pollinator bee *T. angustula*. Potentially, bees from areas with higher percentage of native forest would have lower levels of toxic metals, metalloids and trace elements related to human land use. In addition, the levels of the toxic elements determined in bees would be result of the environmental input/release of these chemicals, which are frequently utilized in activities taking place in most of the classes of land use under study (agriculture, pasture, urban areas, roads). The interaction of these chemical elements with meteorological and geological conditions, allied to their toxicokinetics, will ultimately define the levels found in bees. Therefore, bees foraging areas comprised by activities that involve a significant input of toxic metals/metalloids into the environment such as urban areas and roads or even agriculture are expected to have more toxic elements accumulated, such as Hg, Sb, Zn, Cd, Pb and As. On the other hand, perimeters importantly covered with bare soil and degraded areas, seen as land covers that significantly receive less additive/chemical inputs since they are unproductive, might have lower influence on the levels of toxic metals and metalloids in bees. In addition, we evaluated the potential of *T. angustula* as bioindicators of the environmental quality.

2. Material and methods

2.1. Study area and landscape selection

We conducted this study in the Ecological Corridor Cantareira (south) and Mantiqueira (north) Mountains, in the Atlantic Forest biodiversity hotspot, State of São Paulo, Brazil (Myers et al., 2000). The region has been the focus of many socio-environmental studies, and since 2014 a Long-Term Research has been developed in this corridor (LTER CCM, or *Projeto de Estudo de Longa Duração* in portuguese - PELD

CCM - funded by FAPESP). The altitudes can reach 400 m and the precipitation average is over 1500 mm per year (Mazzei, 2007). The climate of the region, according to the climatic classification of Köeppen, is the humid subtropical type, with the hot and rainy period occurring between October and March. The natural vegetation and land cover of the region are quite heterogeneous, with a strong anthropic pressure. For example, suppression of native vegetation for urban expansion, agriculture, forestry (mainly *Eucalyptus* commercial plantations), tourism, roads, NGO and governmental conservation projects, hunting activities and increasing fires in forest remnants have already been described in this region (Sartorello, 2014).

We created land cover maps from the study area using high-resolution satellite images (Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and/or GIS User Community) of one-meter spatial resolution available in the Basemap extension (ArcGIS 10.2). Within the LTER CCM region, we selected eight regional landscapes with 5 km-radius following a gradient of forest cover ranging from 16% to 69%. To define these landscapes, we used aerial and satellite images from the study region as well as field visits. In addition to forest cover (%) at regional scale, we also calculated Shannon's Index diversity (Shdi), as an estimate for anthropogenic landscape diversity, since most of the classes of land use in the regions under study are resultant of human activity (McGarigal, 2012). Forest cover was calculated using GRASS 6.4

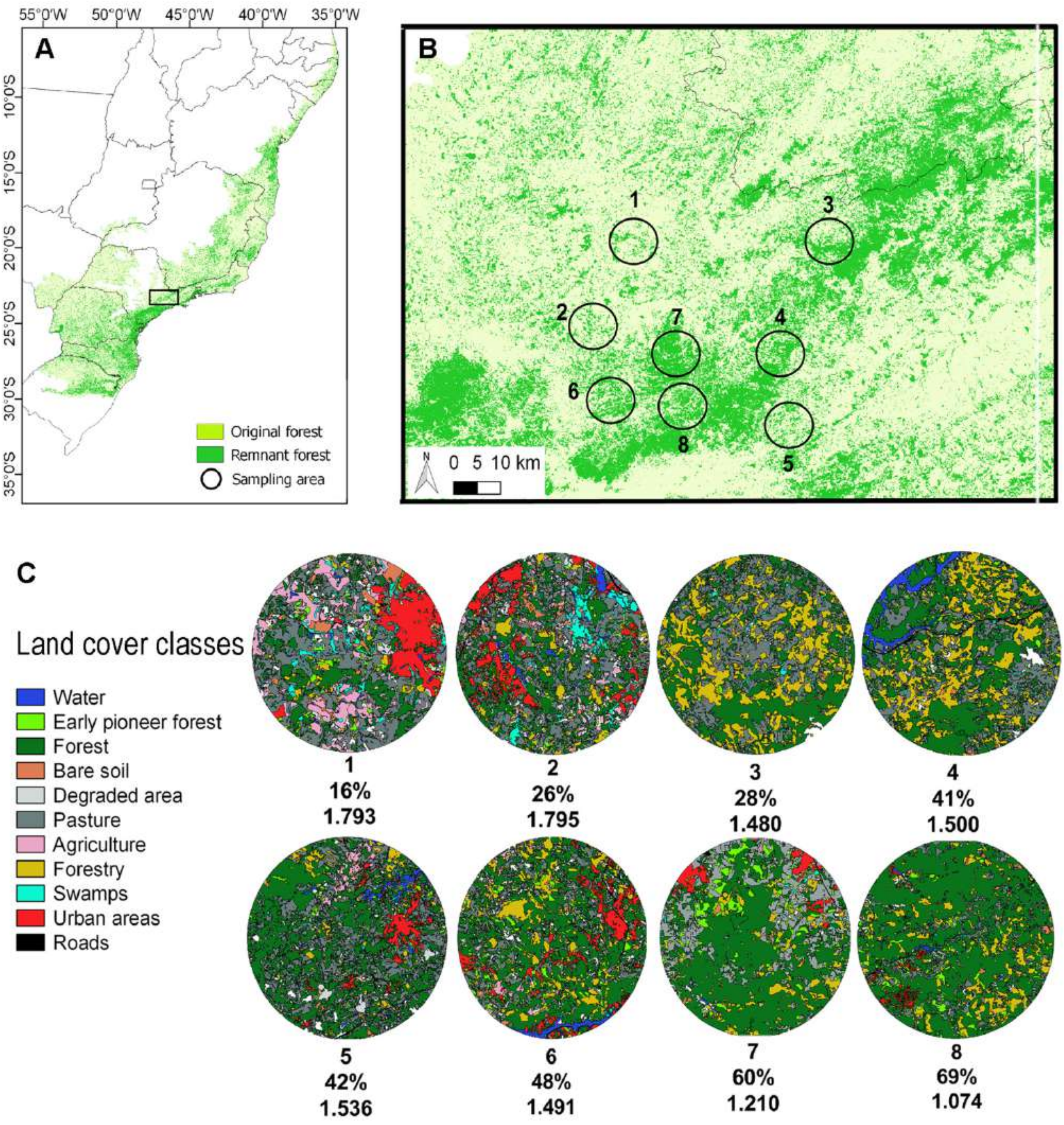

Fig. 1. Sampling landscapes with 5 km radius within Cantareira-Mantiqueira Corridor, situated in the State of São Paulo, Brazil. A) Southern Brazil showing original and remaining forest areas. B) Location of the eight sampling landscapes within the study region. C) Land cover classes of the sampling landscapes; below the landscapes we present native forest cover (%) and spatial heterogeneity (Shannon diversity index). The region is part of the Long-Term Ecological Research at Cantareira-Mantiqueira Corridor (LTER CCM or PELD CCM), São Paulo, Brazil.

(GRASS Development Team, 2015). Landscape heterogeneity and forest cover were calculated within circular areas of 5 km radius surrounding sampling plots. The forest fragments were surrounded by distinct land cover classes (crops such as citrus and coffee, pasture, forestry, urban areas, roads, degraded area, etc.) which combined determined the gradients of landscape heterogeneity (Fig. 1). Detailed information on the classes of land cover per landscape are available in Supplementary Table S1.

2.2. Bee sampling

We installed 70 trap nests (one per tree) (for details, see Caliari-Oliveira et al., 2012) in each of the eight regional landscapes. These traps were placed on tree trunks (nests spaced from each other by at least 50 m distance in random directions) from September 2015 to September 2017 (SISBIO permission number 48402/1, from the Brazilian Ministry of Environment). Every two months, the nests were inspected to verify the presence of individuals. In addition, we performed active searches along the study areas in order to find natural nests (21 natural nests were found, approximately 2–3 per landscape). At least one forager of *Tetragonisca angustula* per occupied colony was collected and individuals were conditioned in metal-free Eppendorf tubes (Eppendorf, NY, USA) labeled with date, time and location. After that, the samples were stored at −20 °C.

2.3. ICP-MS analysis

Samples were weighed and digested with nitric acid (3% v/v) in a closed vessel system at 80 °C for six hours according to the method proposed by Paniz et al. (2018). Subsequently, the resulted digested sample was diluted with Milli-Q water and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). All reagents used were of analytical grade, except nitric acid (HNO_3), which was previously purified in a quartz distiller (Kürner). High purity water (resistivity 18.2 MΩ-cm), obtained from a Milli-Q system (Millipore, Milli-Q Rios, Bedford, MA) was used thoroughly. The plastic and glass containers and flasks were cleaned by immersion in 10% (v/v) HNO_3 for 24 h, washed with Milli-Q water and dried in a laminar flow chamber in a class 1000 room. The determination of the chemical elements was carried out by using an inductively coupled plasma mass spectroscope ELAN DRC II (ICP-MS) (Perkin Elmer Life and Analytical Sciences, USA) and included the analysis of aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), platinum (Pt), rubidium (Rb), antimony (Sb), selenium (Se), strontium (Sr), uranium (U), vanadium (V) and zinc (Zn). The ICP-MS is equipped with a platinum and cone skimmer sampler, both from Perkin Elmer and 99.999% argon (White Martins, SP, Brazil). Stock solutions (10 mg/l) were used for analytical curve preparation (Perkin-Elmer), and rhodium (Rh) was used as an internal standard (10 mg/l). Certified reference materials from the International Atomic Energy Agency (IAEA) were analyzed before and after the analysis of a group of 20 samples for quality control of data.

2.4. Statistical analysis

Chemical elements levels were log10-transformed in order to increase model fitting power. Descriptive statistics was used to summarize the levels of chemical elements accumulated in bees sampled from the eight landscapes. Landscape diversity, including native forest cover and classes of human land use were used as explanatory variables.

We analyzed the influence of native forest and landscape diversity on the concentrations of 21 chemical elements accumulated in bees. To examine landscape diversity (Shdi) or native forest (fixed effect) effects on chemical elements levels determined in *T. angustula* bees, we used linear mixed-effects models (LMEs), fitted by restricted maximum likelihood (REML), from *nlme* package (Pinheiro et al., 2021). Landscape ID and sampling date (month and year) were included as random effects at both models. Despite being part of landscape heterogeneity, the influence of native forest cover has been evaluated separately to emphasize its potential importance and verify a potential beneficial effect on the levels of inorganic contaminants in bees, used as a proxy for assessing bees health which can have an impact in conservation.

We ran a multivariate analysis (Redundancy Analysis) to assess the classes of human land use that better explain the pattern of accumulation of chemical elements in bees. This approach was used since it summarizes the variation in a set of response variables that can be explained by a set of explanatory variables (Legendre and Legendre, 1998). To do that, we used the *rda* function from the vegan package (Oksanen et al., 2015). Agriculture was removed from this analysis due to high multicollinearity (verified by the high Variance Inflation Factor (VIF). Significance of RDA constrained axes was assessed using the ANOVA.cca function and significant axes were then used to identify distribution of samples in relation to landscape covers and metals concentration. We alternatively ran mixed linear models (LMEs) using degraded area, pasture, urban area, road and bare soil as fixed factors (explanatory variables with VIF <3.00 indicated by RDA as major drivers). Landscape ID and sampling date were also included as random effects and all models were fitted by REML (restricted maximum likelihood).

Variance Inflation Factor (VIF) was calculated in order to identify multicollinearity using *vif* function from car package (Fox and Weisberg, 2019). VIF were considered acceptable when lower than 3.00. Estimate, p-values (alpha level $= 0.05$) and t-values were used to determine the direction, significance and magnitude, respectively, of the effect on the concentration found in bees. We performed all statistical analyses in the R environment version 3.4.3 (R-Core Team, 2013).

3. Results and discussion

Human land use has been referred as one of the main threats to biodiversity, leading to extensive forested areas converted into agriculture and pasture fields (Foley et al., 2005; Fierro et al., 2019). Here we investigated the impact of human landscape changes and native forest cover on the accumulation levels of 21 chemical elements in the native social bee *Tetragonisca angustula* in the threatened biome of Atlantic Forest in Brazil. The majority of environmental biomonitoring studies using bees carried out so far has focused on *Apis mellifera* (Fakhimzadeh and Lodenius, 2000; van der Steen et al., 2012; Gutierrez et al., 2015; Costa et al., 2019), mainly due to its widespread geographical distribution, pollination services and consequent economic importance. Even though few of these studies have examined native bee species, our study is unique in evaluating native social stingless bees (Moroń et al., 2014; Exley et al., 2015).

We sampled 214 individuals of *Tetragonisca angustula* from the eight landscapes (27 ± 23 per landscape) along the LTER CCM region. Descriptive data (median and interquartile range) of the toxic and essential elements determined in *T. angustula* bees are summarized in Supplementary Table S2. The medians of toxic and essential elements varied considerably among landscapes, which in turn vary in percentage of native forest, and surrounding land covers. This corroborates the studies of Bromenshenk et al. (1985) and Veleminsky et al. (1990) that have previously suggested that the level of toxic metals in *Appis melifera* bees fluctuates according to their concentration in the environment these insects live in. In our study, the landscape with 41% of forest cover presented the highest medians of the toxic elements such as Hg, Pb, Al, Cr, Pt, U, Sb and As, respectively (Supplementary Table S2). The levels of the elements in bees are either result of environmental inheritance from parent materials or environmental inputs through human activities that might be taking place in this region and their impact needs to be monitored in detail.

3.1. Landscape heterogeneity (primarily human-derived) and native forest cover influence metals levels accumulated in *T. angustula*

In our study region, the more heterogeneous the landscape (e.g., more anthropogenic land cover classes such as pasture, agriculture, urban area, roads, degraded areas, forestry plantations, etc.), the higher the number of potential sources of pollution. These pollution sources may act synergistically considering the accumulation of toxic metals, metalloids and nonmetals in the bees. From the 21 chemical elements studied, we found a positive influence of landscape diversity (which is composed mainly by classes of human land use) on the concentrations of Hg, Zn, Cd and Cr accumulated in *T. angustula* (Table 1). However, for Cu levels, we observed a negative effect of anthropogenic landscape diversity (Table 1). Potentially in one or more of these less heterogeneous landscapes where bees presented higher concentrations of Cu, sources of this element, either natural or anthropogenic, may have increased Cu levels in the environment (Ballabio et al., 2018). Apart from these sources, environmental distribution of Cu is influenced by climatic, geological and pedological factors (Ballabio et al., 2018).

Native forest cover presented a positive effect on the levels of Co and Cu in bees (Table 2). This finding can be related to natural sources of Cu, that include volcanic eruptions, windblown dust, and forest fires (Georgopoulos et al., 2001). Cobalt, in turn, occurs naturally in soils through the breakdown of organic matter, and the weathering of the local minerals into soil particles (Collins and Kinsela, 2010).

An interesting finding is that Hg levels found in bees were directly related to anthropogenic landscape diversity and inversely related to native forest cover (Tables 1 and 2). In addition to Hg, native forest cover (%) presented a significant negative influence on the levels of Cd, Cr and, Zn (Table 2). These results suggest that the proportion of native forest cover does have a protective effect on the environmental concentration of harmful elements with no essential role in the biology of any existing organism, such as Hg and Cd. It is known that vegetation can alter the dispersion of environmental contaminants surrounding roads and urban/industrial areas of emissions of pollutants, which can be subsequently removed from tree canopies by resuspension, rainfall, and leaves fall (Nowak et al., 2013b, 2013a). Trees are widely quoted to be efficient scavengers of particles from the atmosphere, but before the pollutant's removal, some pollinators such as bees can be exposed to significant levels of some toxic metals and metalloids, since atmospheric deposition in the forest interiors is greater than in their edges (Fowler et al., 2004). This phenomenon can be explained by the decreased wind speed and the relatively low pH values - that can increase the bioavailability of metals and chemical elements in soil - seen inside the forest (Raynor et al., 1974; Hutchinson and Whitby, 1977; Pleijel et al., 1996; Takáč et al., 2018). The increased deposition of airborne metal-containing particles, even those originating from distant locations, can offer a considerable risk to bees as well as to other pollinators (van der Steen et al., 2012). A combination of these concepts allied to the characteristics of our study areas can help to understand why some of the elements' levels were inversely correlated with the percentage of native forest cover while others presented a direct relation.

Table 1
Effect of landscape heterogeneity on log10-transformed levels of chemical elements determined in *Tetragonisca angustula* stingless bees sampled from the eight landscapes within the Long-Term Ecological Research Cantareira-Mantiqueira Corridor (LTER CCM/PELD CCM), São Paulo, Brazil.

Chemical element	Estimate	SE	t-value	p-value
Al	0.0844	0.2954	0.2859	0.775
As	-0.8366	0.5256	-1.5915	0.150
Ba	-0.2599	0.4549	-0.5713	0.583
Cd	0.3828	0.1192	3.2101	**0.001*****
Co	-0.1946	0.1786	-1.0901	0.278
Cr	0.6981	0.2212	3.1562	**0.002****
Cu	-0.211	0.0905	-2.3306	**0.021***
Fe	-0.1249	0.4333	-0.2883	0.780
Hg	3.2256	1.1003	2.9317	**0.018***
Mg	0.0255	0.065	0.3929	0.695
Mn	0.0623	0.1257	0.4961	0.620
Ni	-0.3066	0.3009	-1.0189	0.310
Pb	-0.0105	0.1373	-0.0762	0.939
Pt	0.2268	2.3127	0.0981	0.924
Rb	-0.1006	0.3449	-0.2917	0.777
Sb	0.6991	0.4461	1.5672	0.1557
Se	67.442	697.41	0.0967	0.925
Sr	-0.0764	0.3575	-0.2138	0.836
U	0.3991	0.8282	0.4819	0.642
V	0.237	0.3278	0.7231	0.471
Zn	0.2732	0.0868	3.1481	**0.002****

Note: SE, standard error. Sampling date (month and year) and landscape ID were included as random effects. Linear mixed model was fitted by REML (restricted maximum likelihood). Significant relations are indicated by

*** $p \leq 0.001$.
** $p \leq 0.01$.
* $p \leq 0.05$

Table 2
Effect of native forest cover on log10-transformed levels of chemical elements determined in *Tetragonisca angustula* stingless bees sampled from the eight landscapes within the Long-Term Ecological Research Cantareira-Mantiqueira Corridor (LTER CCM/PELD CCM), São Paulo, Brazil.

Chemical element	Estimate	SE	t-value	p-value
Al	0.0018	0.0117	0.152	0.882
As	0.0111	0.0074	1.4855	0.175
Ba	-0.0001	0.0065	-0.0212	0.983
Cd	-0.0039	0.0018	-2.137	**0.035***
Co	0.0084	0.0029	2.9436	**0.004****
Cr	-0.0071	0.0038	-1.8754	**0.043***
Cu	0.0043	0.0011	3.8077	**<<0.0001*****
Fe	0.0016	0.006	0.2587	0.802
Hg	-0.0437	0.0158	-2.7584	**0.024***
Mg	0.0001	0.0008	0.1716	0.864
Mn	-0.0018	0.0042	-0.4174	0.687
Ni	0.0112	0.0098	1.1415	0.286
Pb	0.0029	0.0058	0.5024	0.628
Pt	-0.0023	0.0322	-0.0727	0.943
Rb	0.0042	0.0046	0.9291	0.380
Sb	-0.0099	0.0062	-1.5957	0.149
Se	2.3783	1.2933	1.8389	0.068
Sr	-0.0764	0.005	9E-05	0.999
U	-0.0035	0.0116	-0.2978	0.773
V	0.0006	0.0141	0.0454	0.964
Zn	-0.0033	0.0013	-2.5317	**0.035***

Note: SE, standard error. Sampling date (month and year) and landscape ID were included as random effects. Model was fitted by REML (restricted maximum likelihood). Significant relations are indicated by

* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

However, our results only partially corroborate with our predictions regarding native forest cover, since it presented a significant negative relation with only a few of well-known toxic elements. The remaining chemical elements neither show a significant relation with the percentage of forest cover nor with the landscape heterogeneity (Tables 1 and 2). More studies are needed to better comprehend the impact of vegetation on the environmental mobility and availability of toxic metals and metalloids.

3.2. Effect of human land use classes on toxic metals accumulation in *T. angustula*

We ran a Redundancy Analysis (RDA) to further explore the influence of the classes of human land use on metals and metalloids' levels found in bees (Fig. 2). The RDA model explained 26.04% (constrained) of the total variance. Only the first two constrained axes from RDA analyses were significant (p-value from RDA1 = 0.001; p-value RDA2 = 0.006) to explain the concentration of metals found on bees. This means

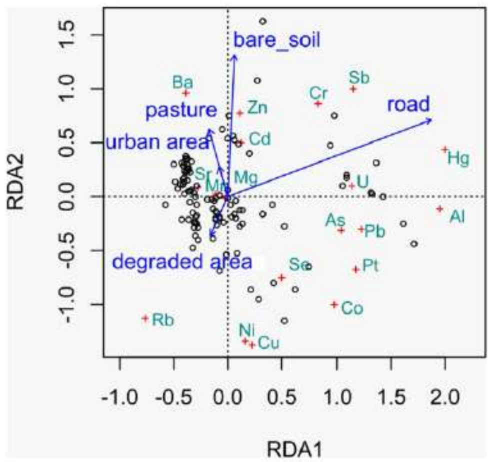

Fig. 2. Redundancy analysis (RDA) showing the variation of chemical elements levels being explained by anthropogenic landscape covers.
Footnote: The empty black circles correspond to sampling units; the red positive symbols correspond to the chemical elements; and the blue arrows are the predictors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that anthropogenic land covers explain 26.04% of the variation observed in chemical elements found in bees. This proportion is mainly explained by road and bare soil, since these variables most contributed to the first and second axes, respectively. For instance, road presented the most important contribution to the first axis and the elements most associated with this landscape cover were Cr, Hg, Sb Al, U, As, Pb and Pt. These elements are components of crustal soil, and predominantly attributed to resuspended road dust (Goryainova et al., 2016). Traffic-related emissions, such as those containing Sb, are one of the main sources of soil pollution. The levels of this metalloid in the soil have been recently demonstrated to decrease with increasing distance from roads being also strongly linked with brake lining sources (where it has been used as a substitute for the banned asbestos) (Földi et al., 2018). Cr and Pb have been also discussed as coming from vehicular source in a previous biomonitoring study (da Silveira Fleck et al., 2017). Considering Cr in soils, it is known that it can be also result of the natural geological substrate degradation process as well as to the use of substances containing it, such as pesticides and fertilizers (Ertani et al., 2017). It is believed that most Cr in soil is in the trivalent form (less toxic), complexed with mineral structures (Branca et al., 1990). Even though we did not identify the amount of the different Cr species (III or VI) in bees, our results show that roads are significantly related to environmental Cr input and ultimately to accumulated Cr in bees. On the other hand, concentrations of Zn, Cd, Mn, Mg, Ba and Sr were associated mainly with bare soil, yet pasture and urban area also centered an important number of bees presenting significant amounts of these elements on their bodies. Moreover, Rb levels were shown to be associated with the presence of degraded area, what corroborates its natural presence in some types of soils (Tyler, 1983).

Our alternative strategy (mixed linear model), performed to verify whether combined classes of human land use (indicated by the RDA) influence the levels of chemical elements in bees (Table 3) showed that urban area, road, degraded area, bare soil and pasture are anthropogenic landcovers that significantly explain the level of Cd, Hg and Zn found in bees (Table 3). This finding corroborates with a recent study that showed, through the evaluation of Cd in soil samples, that road accessibility is a proxy for the spatial heterogeneity of urbanization (Qiu et al., 2019). According to the authors, oscillations in the Cd levels in soil, together with the road accessibility gradient must be considered in the formulation of targeted policies for controlling Cd contamination in countrified areas. In addition, it is known that some fertilizers used in pastures may contain several contaminants, of which Cd is considered to be of high concern (Loganathan et al., 2008).

Table 3
Effect of the combined classes of human land use on the log10-transformed concentrations of chemical elements determined in *Tetragonisca angustula* bees sampled from the eight landscapes within the Long-Term Ecological Research Cantareira-Mantiqueira Corridor (LTER CCM/PELD CCM), São Paulo, Brazil.

Chemical element	Fixed factor	Estimate	t-value	SE	p-value
Al	*Degraded area/Bare soil/Pasture/Road/Urban area*	-0.0001	0.0002	-0.402	0.698
As		-0.0002	0.0001	-1.707	0.126
Ba		0.0000	0.0001	0.301	0.771
Cd		0.0002	0.0001	2.146	**0.046***
Co		-0.0002	0.0001	-1.193	0.267
Cr		0.0002	0.0002	1.202	0.264
Cu		-0.0001	0.0001	-0.828	0.432
Fe		0.0000	0.0001	-0.361	0.727
Hg		0.0009	0.0003	2.872	**0.021***
Mg		0.0001	0.0001	0.602	0.564
Mn		0.0001	0.0001	0.804	0.444
Ni		-0.0001	0.0002	-0.604	0.562
Pt		0.0003	0.0006	0.388	0.709
Rb		-0.7860	1.9410	-0.405	0.696
Sb		0.0002	0.0001	1.804	0.109
Se		0.0924	0.1946	0.475	0.648
Sr		0.0001	0.0001	0.605	0.562
U		-0.0003	0.0007	-0.351	0.735
V		-0.0310	0.0849	-0.365	0.724
Zn		0.0001	0.0000	3.219	**0.012***

Note: SE, standard error. Sampling date (month and year) and landscape ID were included as random effects. Model was fitted by REML (restricted maximum likelihood). Significant relations are indicated by
* $p \leq 0.05$.

Few studies have identified a relation between the levels of Hg in bees and croplands (Toporcak et al., 1992; Zarić et al., 2018). Even though Hg is liberated into the environment from natural and anthropogenic sources, coal-fired power plant has been identified as the largest source of Hg emissions (Li et al., 2017). Apparently, the use of organic fertilizers, such as sewage sludges and municipal solid wastes, pesticides in addition to industrial and urban emissions (followed by Hg deposition) are the most common sources of Hg inputs into the environment (Sánchez et al., 2017; Sundseth et al., 2017). We are not aware of any coal-fired power plants operation in the region. On the other hand, it is also important to consider that the presence of Hg in soil, plant litter, and sediments is a function of geochemical cycles and specific variables, such as climate, land usage, vegetal cover, as well as physical, chemical, and biological processes that take place in this environment (Demers et al., 2007; Bonotto et al., 2018; Ma et al., 2019). The information above is important to be considered since we also saw degraded areas as a factor explaining the levels of this toxic metal in bees (Table 3). In addition to Hg, farmlands can have high levels of other toxic metals such as Zn (Li et al., 2019). It is known that agricultural practices such as the use of organic and inorganic fertilizers, pesticides, fungicides, as well as sewage sludge and wastewater, also can add a good amount of Zn (among other metals) into the soil and water ecosystems (Nagajyoti et al., 2010). Additionally, Zn can also be released in the atmosphere by vehicular traffic (i.e., tire wear emissions, antioxidant in engine oils) (da Silveira Fleck et al., 2017).

Exposure to toxic elements may impact pollinators' health as well as their ability to survive within human dominated landscapes (Moroń et al., 2014). Individual growth, behavior, and survival, as well as the reduction of abundance and/or diversity of assemblages are examples of processes that are affected by pollution (Sheehan, 1984). Environmental inputs of metals seen accumulated in bees in this study such as Pb, Cd or Cu can result in neurotoxicity (Nikolić et al., 2019) and also can limit the availability of plant resources used as food and material to build nests by bees (Burden et al., 2019). The contamination of the environment by toxic metals has an additional indirect harmful effect on certain bees' populations, being associated with decreased number of brood cells and increasing offspring mortality (Moroń et al., 2014). Thus, in addition to their importance for environmental quality, highly forested areas play a pivotal role as source of nutrition and nest material that can protect bees from exposure and accumulation of highly toxic metals such as Hg, Cd and Cr (Table 2). Additionally, bees preferentially explore habitats inside forest fragments (Newton et al., 2018), being less exposed to urban or human effects. Therefore, we believe that the levels of the toxic metals found in the bees in this study may be even an underestimation on the levels existing in the environmental surroundings (soil, air and water) within the LTER region, given this behavior of bees.

3.3. Comparison of toxic metals/metalloids levels with the literature

We made a summary of available information on the levels of toxic metals/metalloids determined in *T. angustula* (this study) and several other bee species carried through biomonitoring studies developed worldwide (Table 4). The great majority of studies have been performed using *Apis mellifera* (which is an exotic species in many regions, as it is in Brazil), what highlights the importance to carry out studies using native bees like *T. angustula*.

The levels of Hg in our study were lower than those reported in *A. mellifera* bees collected in industrially contaminated areas in Slovakia (Toporcak et al., 1992). However, the range of Hg levels (0.022 to about 427 ng/g) determined in our study is considerably wider than that reported in honeybees collected from an urban-industrial area, which ranged from 100 to 250 ng/g (Zarić et al., 2018). These authors showed that the highest levels of Hg in honeybees were found nearby a petrochemical plant, whereas the lowest levels were showed in bees sampled from rural sites, nearby environmental protected areas. This same pattern was observed in our study, where the levels of Hg were much lower in areas with higher forest cover (> 60%, Supplementary Table S2) in comparison to those with lower percentage (< 41%, Supplementary Table S2). However, considering the maximum Hg level reported in this study (Supplementary Table S2), it is still a matter of concern, since exposure to this toxic metal can generate physiological disturbances also in insects, such as bees, as previously reported (Gizaw et al., 2020).

The levels of Cd, Cr, and Sb determined in *T. angustula* bees were much lower in comparison to those detected in honeybees in the study of Porrini et al. (2003) (Table 4). For Al, a metal associated with neurotoxic effects, the levels found in *T. angustula* bees in this study were higher than those reported by Silici et al. (2016) and by Zarić et al. (2017) in *A. mellifera* bees. Among the areas studied by Zarić et al. (2017), bees from a region with agricultural activities and natural vegetation presented the lowest level of Al. In this same context, Exley et al. (2015) found that bumblebee pupae were also considerably contaminated with Al, which generated adverse effects, since the pupae tended to be smaller when exposed to high Al levels. Pollen and nectar were the suspected sources of bees' contamination. Also, for Pb, *T. angustula* bees showed higher levels in comparison to the *A. mellifera* bees from a Turkish area situated at least 10 km distant from a thermal power plant (Silici et al., 2016).

Since elements determined in *T. angustula* showed to be significantly affected by native forest and human-modified landscapes, we suggest that this native bee can serve as an important bioindicator of environmental quality in the Atlantic Forest. *T. angustula* is known to have a shorter flight range, and the chemical elements concentrations accumulated potentially refer to environmental conditions within fine scale range (1 km radius) (Araujo et al., 2004). This stingless bee species has generalist habits and can build their nests at different locations, including urban areas and visit a wide variety of crops. Due to its behavior and according to our results, the levels of inorganic contaminants in *T. angustula* can serve as a proxy for assessing bee health and impacts on conservation, as well as to estimate the environmental quality of different areas.

4. Conclusion

Within human-dominated landscapes, where different anthropogenic land covers take place, it is extremely important to evaluate how these classes of human land use influence the levels of toxic chemical elements, particularly in pollinators. Although by varying land cover we can increase spatial heterogeneity, depending on its cover classes (%) we will have positive or negative effects on species abundance and diversity. Regarding ecotoxicity - as is the case of toxic elements levels accumulated in *T. angustula* bees – the results are also dependent on the context of land use. Here we present a beneficial effect of forested areas on the concentrations of some well-known toxic elements accumulated in bees (Hg, Cd, and Cr), stressing the important role of ecological corridors, especially surrounding agricultural and urban areas, as well as increasing green areas inside urban regions. Anthropogenic land cover classes, especially road, urban area, pasture and bare soil appear to be good predictors of the accumulation of specific metals and metalloids in bees, although some results were contrary to our expectations. For instance, one can think of bare soil as an area of little imputation of chemical additives or even unproductivity, but for potential toxic elements such as Zn, Cd, Ba and Sr, this land cover was pointed to participate as an important driver of their levels in bees. Since inorganic chemical elements are not degraded through time, both current as well as past activities using additives/chemicals, together with meteorological and geological conditions and individual factors, may be potential explanations for having this unproductive area as driver of accumulated elements in bees (for instance, unproductive areas with resuspended soil that once had mining, agriculture, pasture, etc.).

By considering that pollinators critically contribute to global food production (Garibaldi et al., 2011; Giannini et al., 2015), and that it is crucial to understand how these species respond to anthropogenic changes in land cover, our study sheds light on how land covers (native forest and human-modified) can influence the levels of contaminants in insects within human-dominated landscapes. Also, our data reinforce the potential use of *T. angustula* bees as bioindicators of environmental quality and also show that these organisms are being directly affected by anthropogenic activities through different land uses within an ecological corridor, offering potential risks for the Neotropical ecosystem. The generation of predictions of the levels of toxic metals and metalloids based on land use can both contribute to friendly farming planning as well as to support public policy development on the surrounding of protected areas and biodiversity conservation hotspots.

Funding

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (to M.M.B.), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2016/14737-3 to A.C.C.F. and FAPESP 2016/07661-0 to M.F.H.C.) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant number 149154/2018-6 to D.A.A.). M.C.R. thanks FAPESP (processes #2013/50421-2; #2020/01779-5), CNPq (processes #312045/2013-1; #312292/2016-3) and PROCAD/CAPES (project #88881.068425/2014-01) for their financial support.

CRediT authorship contribution statement

Marcela de Matos Barbosa: Conceptualization, Methodology, Investigation, Software, Formal analysis, Validation, Visualization, Writing - original draft preparation, Writing - review & editing. **Ana Carolina Coelho Fernandes:** Investigation, Formal analysis, Visualization. **Rafael Souza Cruz Alves:** Investigation. **Denise A. Alves:** Writing - original draft preparation, Resources. **Fernando Barbosa Junior:** Investigation, Resources. **Bruno Lemos Batista:** Investigation, Resources. **Milton Cezar Ribeiro:** Conceptualization, Methodology, Software, Writing - original draft preparation, Writing - review &

Table 4
Comparison of the levels of toxic metals and metalloids in *T. angustula* with results obtained in studies developed worldwide.

Chemical element	Arithmetic mean ± SD (min–max)	Bee species	Country - Study	Sampling sites	Sample type
Al (μg/g)	32.44 ± 43.96 (1.61 – 206.03)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor of Atlantic Forest	forager
	3.48 ± 0.16	Apis mellifera	Turkey (Silici et al., 2016)	Samples located 10–22 km away from thermal power plants	forager
	104 ± 40[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	16.8 ± 5.4[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	66 ± 26[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	8.4 ± 3.9[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	18.2–60.0	Bombus terrestris audax	Belgium (Exley et al., 2015)	min-max found in a rural area	pupae
	16.3–149.4	Bombus terrestris audax	Belgium (Exley et al., 2015)	min-max found in an urban area	pupae
As (ng/g)	22.61 ± 16.7 (1.06–101.7)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	0.13–1.64	Apis mellifera	Netherlands (van der Steen et al., 2016)	Pooled samples from agricultural, woods and urban areas	worker
	0.67–0.83	Apis mellifera	Netherlands (van der Steen et al., 2012)	An urban area with cement industry and the glass industry. Colonies located in the city center	worker
	0.40 ± 0.24	Apis mellifera	Serbia (Zarić et al., 2018)	Industrial area (presence of two thermal power plants)	forager
	0.28 ± 0.16	Apis mellifera	Serbia (Zarić et al., 2018)	Urban area	forager
	0.206 ± 0.089	Apis mellifera	Serbia (Zarić et al., 2018)	Petrochemical industry	forager
	0.19 ± 0.13	Apis mellifera	Serbia (Zarić et al., 2018)	Suburban area	forager
	0.027 ± 0.053	Apis mellifera	Italy (Giglio et al., 2017)	Urban area	forager
	0.050 ± 0.018	Apis mellifera	Italy (Giglio et al., 2017)	Urban area	forager
	0.195 ± 0.036	Apis mellifera	Serbia (Zarić et al., 2018)	The rural area bordering protected area	forager
Ba (μg/g)	5.83 ± 6.5 (0.72–55.9)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	3.21 ± 0.50[a]	Apis mellifera	Serbia (Zarić et al., 2017)	Urban area with significant agriculture activity	forager
	1.16 ± 0.72[a]	Apis mellifera	Serbia (Zarić et al., 2017)	Urban area with significant agriculture activity	forager
	1.86 ± 0.33[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	0.46 ± 0.11[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
Cd (ng/g)	14.7 ± 11.24 (4.11–92.3)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	0.45 7.66	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants)	forager
	100,170	Apis mellifera	Poland (Roman, 2005)	Industrial area	forager
	80,195	Apis mellifera	Italy (Costa et al., 2019)	Urban area	forager
	140,160	Apis mellifera	Poland (Roman, 2005)	Agricultural-forest region	forager
	28,704,230	Apis mellifera	Italy (Costa et al., 2019)	Areas in city center or near highway	worker
	50,750	Apis mellifera	Netherlands- (van der Steen et al., 2012)	An urban area with cement industry and glass industry. Colonies located in city center	worker
	50,730	Apis mellifera	Netherlands (van der Steen et al., 2016)	Pooled samples from agricultural, woods and urban areas	worker
	265 ± 31[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	57 ± 16[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	312 ± 16[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	86 ± 62[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	< 1.0	Osmia rufa	Poland- (Moroń et al., 2014)	Urban area	adult female
	< 1.0	Osmia rufa	United Kingdom (Moroń et al., 2014)	Urban area	adult female
Cr (ng/g)	89.56 ± 94.14 (5.38–488.5)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	3.88 10.7	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	116.00– 520	Apis mellifera	Italy (Costa et al., 2019)	Areas in city center or near highway	worker
	160–230	Apis mellifera	Poland (Roman, 2005)	Industrial area	forager
	120,180	Apis mellifera	Poland (Roman, 2005)	Agricultural-forest region	forager
	190–1420	Apis mellifera	Netherlands- (van der Steen et al., 2016)	Pooled samples from agricultural, woods and urban areas	worker
	150,280	Apis mellifera	Netherlands- (van der Steen et al., 2012)	Urban area with cement industry and glass industry. Colonies located in city center	worker
	380 ± 100[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	52 ± 36[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	610 ± 420[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	90 ± 40[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
Cu (μg/g)	11.01 ± 5.13 (2.97–31.83)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	5.94 10.4	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	28.914 ± 0.071[a]	Apis mellifera	Serbia (Zarić et al., 2017)	Urban area with significant agriculture activity	forager
	16.4 ± 3.3[a]	Apis mellifera	Serbia (Zarić et al., 2017)	Urban area with significant agriculture activity	forager
	32.0 ± 2.9[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	15.6 ± 4.1[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	11.65 19.77	Apis mellifera			worker

(continued on next page)

Table 4 (*continued*)

Chemical element	Arithmetic mean ± SD (min–max)	Bee species	Country - Study	Sampling sites	Sample type
			Netherlands- (van der Steen et al., 2012)	An urban area with cement industry and the glass industry. Colonies located in the city center	
	12.87 20.84	Osmia bicornis	Poland (Filipiak, 2019)	Experimental garden	adult female
	9.59 17.92	Osmia bicornis	Poland (Filipiak, 2019)	Experimental garden	adult male
Fe (μg/g)	52.97 ± 34.15 (14.3–186.5)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	49.2 102	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	174 ± 17[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	90 ± 15[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	211 ± 11[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	114 ± 49[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	96.18 119.82	Osmia bicornis	Poland (Filipiak, 2019)	Experimental garden	adult female
Hg (ng/g)	43.6 ± 84.33 (0.022–427.02)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
					forager
	45 ± 40	Apis mellifera	Serbia (Zarić et al., 2018)	Industrial area (presence of two thermal power plants)	forager
	70 ± 32	Apis mellifera	Serbia (Zarić et al., 2018)	Urban area	forager
	118 ± 74	Apis mellifera	Serbia (Zarić et al., 2018)	Near to petrochemical industry	forager
	115 ± 80	Apis mellifera	Serbia (Zarić et al., 2018)	Suburban area	forager
	26 ± 36	Apis mellifera	Serbia (Zarić et al., 2018)	The rural area bordering protected area	forager
Ni (ng/g)	529.2 ± 772.3 (9.73–5542)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	12.8 310	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	2,481,396	Apis mellifera	Italy (Costa et al., 2019)	Urban area	forager
	270,420	Apis mellifera	Poland (Roman, 2005)	Agricultural-forest region	forager
	360,910	Apis mellifera	Poland (Roman, 2005)	Industrial area	forager
	1030 ± 220[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	235 ± 60[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	1030 ± 222[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	210 ± 110[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	190,470	Apis mellifera	Netherlands (van der Steen et al., 2012)	An urban area with cement industry and glass industry. Colonies located in city center	worker
Pb (ng/g)	113.32 ± 85.77 (24.87–599.09)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	1,901,670	Apis mellifera	Netherlands- (van der Steen et al., 2012)	An urban area with cement industry and the glass industry. Colonies located in the city center	worker
	4.01 24.1	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	61.00 125	Apis mellifera	Italy (Conti and Botrè, 2001)	Areas in city center or near highway	worker
	< 1.0	Osmia rufa	Poland- (Moroń et al., 2014)	Urban area	adult female
	< 1.0	Osmia rufa	United Kingdom (Moroń et al., 2014)	Urban area	adult female
Sb (ng/g)	4.78 ± 4.76 (0.74 31.66)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	52 ± 19	Apis mellifera	Serbia (Zarić et al., 2018)	Urban area	forager
	25 ± 10	Apis mellifera	Serbia (Zarić et al., 2018)	Petrochemical industry	forager
	16 ± 10	Apis mellifera	Serbia (Zarić et al., 2018)	Suburban area	forager
	21.9 ± 2.4	Apis mellifera	Serbia (Zarić et al., 2018)	The rural area bordering protected area	forager
	20.6 ± 3.6	Apis mellifera	Serbia (Zarić et al., 2018)	Industrial area (presence of two thermal power plants)	forager
Zn (μg/g)	75.18 ± 94.35 (34.68–996.05)	Tetragonisca angustula	Brazil (current study)	5 km radius-landscapes within an ecological corridor	forager
	8.56 16.6	Apis mellifera	Turkey (Silici et al., 2016)	Industrial area (near power plants) (values refer to the minimum and maximum mean values from 10 sampled areas)	forager
	61.14 100.64	Apis mellifera	Netherlands- (van der Steen et al., 2012)	An urban area with cement industry and glass industry. Colonies located in city center	worker
	56.60 170	Apis mellifera	Netherlands- (van der Steen et al., 2016)	Pooled samples from agricultural, woods and urban areas	worker
	151.1 ± 1.5[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	102 ± 46[a]	Apis mellifera	Serbia (Zarić et al., 2017)	An urban area with significant agriculture activity	forager
	143 ± 18[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The highest level in urban area	forager
	74 ± 13[a]	Apis mellifera	Serbia (Zarić et al., 2017)	The lowest level in urban area	forager
	59,100	Apis mellifera	Finland (Fakhimzadeh and Lodenius, 2000)	Industrial area (values refer to the minimum and maximum mean values from 4 sampled areas)	forager
	6181	Apis mellifera	Finland (Fakhimzadeh and Lodenius, 2000)	Urban area (values refer to the minimum and maximum mean values from 4 sampled areas)	forager
	52.5 76.2	Apis mellifera	Czechoslovakia (Leita et al., 1996)	Colonies located 50 m from the extra urban crossroad	forager
	153.34 204.4	Apis mellifera	Czechoslovakia (Veleminsky et al., 1990)	Industrial area	forager

(*continued on next page*)

Table 4 (*continued*)

Chemical element	Arithmetic mean ± SD (min–max)	Bee species	Country - Study	Sampling sites	Sample type
	88.0 ± 3.3	Osmia rufa	Poland- (Moroń et al., 2014)	The lowest level found in urban area	adult female
	113.0 ± 4.8	Osmia rufa	Poland- (Moroń et al., 2014)	The highest level found in urban area	adult female
	55.9 ± 4.0	Osmia rufa	United Kingdom (Moroń et al., 2014)	The lowest level found in urban area	adult female
	103.0 ± 3.1	Osmia rufa	United Kingdom (Moroń et al., 2014)	The highest level found in urban area	adult female
	158.31 235.08	Osmia bicornis	Poland (Filipiak, 2019)	Experimental garden	adult female

[a] Note: of dry weight samples. SD, standard deviation. min, minimum concentration found. max, maximum concentration found.

editing, Resources, Visualization. **Maria Fernanda Hornos Carneiro:** Conceptualization, Methodology, Validation, Visualization, Formal analysis, Writing - original draft preparation, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are especially thankful to Roberto Gaioski Jr. for field assistance, to Leonardo Trevellin for statistical support and to private landowners that permitted sampling bees from their lands.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112147.

References

Agnan, Y., Séjalon-Delmas, N., Claustres, A., Probst, A., 2015. Investigation of spatial and temporal metal atmospheric deposition in France through lichen and moss bioaccumulation over one century. Sci. Total Environ. 529, 285–296. https://doi.org/10.1016/j.scitotenv.2015.05.083.

Ali, W., Aslam, M.W., Feng, C., Junaid, M., Ali, K., Li, S., Chen, Z., Yu, Z., Rasool, A., Zhang, H., 2019. Unraveling prevalence and public health risks of arsenic, uranium and co-occurring trace metals in groundwater along riverine ecosystem in Sindh and Punjab, Pakistan. Environ. Geochem. Health 41 (5), 2223–2238. https://doi.org/10.1007/s10653-019-00278-7.

Araujo, E.D., Costa, M., Chaud-Netto, J., Fowler, H.G., 2004. Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. Braz. J. Biol. 64, 563–568. https://doi.org/10.1590/S1519-69842004000400003.

Azad, A.M., Frantzen, S., Bank, M.S., Johnsen, I.A., Tessier, E., Amouroux, D., Madsen, L., Maage, A., 2019. Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway. Sci. Total Environ. 667, 622–637. https://doi.org/10.1016/j.scitotenv.2019.02.352.

Azam, I., Afsheen, S., Zia, A., Javed, M., Saeed, R., Sarwar, M.K., Munir, B., 2015. Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. BioMed Res. Int. 2015, 1–11. https://doi.org/10.1155/2015/942751.

Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P., Montanarella, L., 2018. Copper distribution in European topsoils: an assessment based on LUCAS soil survey. Sci. Total Environ. 636, 282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268.

Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313, 351–354. https://doi.org/10.1126/science.1127863.

Bilandžić, N., Tlak Gajger, I., Kosanović, M., Čalopek, B., Sedak, M., Solomun Kolanović, B., Varenina, I., Luburić, Đ.B., Varga, I., Đokić, M., 2017. Essential and toxic element concentrations in monofloral honeys from Southern Croatia. Food Chem. 234, 245–253. https://doi.org/10.1016/j.foodchem.2017.04.180.

Bonotto, D.M., Wijesiri, B., Vergotti, M., da Silveira, E.G., Goonetilleke, A., 2018. Assessing mercury pollution in Amazon river tributaries using a Bayesian network approach. Ecotoxicol. Environ. Saf. 166, 354–358. https://doi.org/10.1016/j.ecoenv.2018.09.099.

Boscolo, D., Tokumoto, P., Ferreira, P., Ribeiro, J., Silveira dos Santos, J., 2017. Positive responses of flower visiting bees to landscape heterogeneity depend on functional connectivity levels. Perspect. Ecol. Conserv. 15, 18–24. https://doi.org/10.1016/j.pecon.2017.03.002.

Branca, M., Dessi, A., Kozlowski, H., Micera, G., Swiatek, J., 1990. Reduction of chromate ions by glutathione tripeptide in the presence of sugar ligands. J. Inorg. Biochem. 39, 217–226. https://doi.org/10.1016/0162-0134(90)84005-A.

Bromenshenk, J.J., Carlson, S.R., Simpson, J.C., Thomas, J.M., 1985. Pollution monitoring of puget sound with honey bees. Science 227, 632–634. https://doi.org/10.1126/science.227.4687.632.

Burden, C.M., Morgan, M.O., Hladun, K.R., Amdam, G.V., Trumble, J.J., Smith, B.H., 2019. Acute sublethal exposure to toxic heavy metals alters honey bee (Apis mellifera) feeding behavior. Sci. Rep. 9, 4253. https://doi.org/10.1038/s41598-019-40396-x.

Caliari-Oliveira, R., Menezes, C., Soares, E., Imperatriz-Fonseca, V.L., 2012. Trap-nests for stingless bees (Hymenoptera, Meliponini). Apidologie 44. https://doi.org/10.1007/s13592-012-0152-y.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T. L., 2011. Patterns of widespread decline in North American bumble bees. Proc. Natl. Acad. Sci. U.S.A. 108, 662–667. https://doi.org/10.1073/pnas.1014743108.

Cane, J.H., 2001. Habitat fragmentation and native bees: a premature verdict? Conserv. Ecol. 5, 3. https://doi.org/10.5751/ES-00265-050103.

Carneiro, M.F., Ribeiro, F.Q., Fernandes-Filho, F.N., Lobo, D.J.A., Rhoden, C.R., Mauad, T., Saldiva, P.H.N., Carvalho-Oliveira, R., 2011. Pollen abortion rates, nitrogen dioxide by passive diffusive tubes and bioaccumulation in tree barks are effective in the characterization of air pollution. Environ. Exp. Bot. 72, 272–277. https://doi.org/10.1016/j.envexpbot.2011.04.001.

Cavanagh, J.E., Zawar-Reza, P., Wilson, J.G., 2009. Spatial attenuation of ambientparticulate matter air pollution within an urbanised native forest patch. Urban For. Urban Green. 8, 21–30. https://doi.org/10.1016/j.ufug.2008.10.002.

Chara-Serna, A.M., Epele, L.B., Morrissey, C.A., Richardson, J.S., 2019. Nutrients and sediment modify the impacts of a neonicotinoid insecticide on freshwater community structure and ecosystem functioning. Sci. Total Environ. 692, 1291–1303. https://doi.org/10.1016/j.scitotenv.2019.06.301.

Collins, R.N., Kinsela, A.S., 2010. The aqueous phase speciation and chemistry of cobalt in terrestrial environments. Chemosphere 79, 763–771. https://doi.org/10.1016/j.chemosphere.2010.03.003.

Conti, M.E., Botrè, F., 2001. Honeybees and their products as potential bioindicators of heavy metals contamination. Environ. Monit. Assess. 69, 267–282. https://doi.org/10.1023/A:1010719107006.

Corbi, J.J., Froehlich, C.G., Trivinho-Strixino, S., Dos Santos, A., 2011. Evaluating the use of predatory insects as bioindicators of metals contamination due to sugarcane cultivation in neotropical streams. Environ. Monit. Assess. 177, 545–554. https://doi.org/10.1007/s10661-010-1655-5.

Costa, A., Veca, M., Barberis, M., Tosti, A., Notaro, G., Nava, S., Lazzari, M., Agazzi, A., Maria Tangorra, F., 2019. Heavy metals on honeybees indicate their concentration in the atmosphere. A proof of concept. Ital. J. Anim. Sci. 18, 309–315. https://doi.org/10.1007/s10661-010-1655-5.

da Silveira Fleck, A., Carneiro, M.F.H., Barbosa, F., Amantea, S.L., Rhoden, C.R., 2017. The use of tree barks and human fingernails for monitoring metal levels in urban areas of different population densities of Porto Alegre, Brazil. Environ. Sci. Pollut. Res. 24, 2433–2441. https://doi.org/10.1007/s11356-016-7832-5.

De Nicola, F., Maisto, G., Prati, M.V., Alfani, A., 2008. Leaf accumulation of trace elements and polycyclic aromatic hydrocarbons (PAHs) in Quercus ilex L. Environ. Pollut. 153, 376–383. https://doi.org/10.1016/j.envpol.2007.08.008.

Demers, J.D., Driscoll, C.T., Fahey, T.J., Yavitti, J.B., 2007. Mercury cycling in litter and soil in different forest types in the Adirondack region, New York, USA. Ecol. Appl. 17, 1341–1351. https://doi.org/10.1890/06-1697.1.

Eeva, T., Sorvari, J., Koivunen, V., 2004. Effects of heavy metal pollution on red wood ant (Formica s. str.) populations. Environ. Pollut. 132, 533–539. https://doi.org/10.1016/j.envpol.2004.05.004.

Ertani, A., Mietto, A., Borin, M., Nardi, S., 2017. Chromium in agricultural soils and crops: a review. Water Air Soil Pollut. 228, 190. https://doi.org/10.1007/s11270-017-3356-y.

Esposito, F., Memoli, V., Di Natale, G., Trifuoggi, M., Maisto, G., 2019. Quercus ilex L. leaves as filters of air Cd, Cr, Cu, Ni and Pb. Chemosphere 218, 340–346. https://doi.org/10.1016/j.chemosphere.2018.11.133.

Exley, C., Rotheray, E., Goulson, D., 2015. Bumblebee pupae contain high levels of aluminium. PLoS One 10, e0127665. https://doi.org/10.1371/journal.pone.0127665.

Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34, 487–515. https://doi.org/10.1146/annurev.ecolsys.34.011802.132419.

Fakhimzadeh, K., Lodenius, M., 2000. Heavy metals in finnish honey, pollen and honey bees. Apiacta 35, 85–95.

Fierro, P., Valdovinos, C., Arismendi, I., Díaz, G., Jara-Flores, A., Habit, E., Vargas-Chacoff, L., 2019. Examining the influence of human stressors on benthic algae, macroinvertebrate, and fish assemblages in Mediterranean streams of Chile. Sci. Total Environ. 686, 26–37. https://doi.org/10.1016/j.scitotenv.2019.05.277.

Filipiak, M., 2019. Key pollen host plants provide balanced diets for wild bee larvae: a lesson for planting flower strips and hedgerows. J. Appl. Ecol. 56, 1410–1418. https://doi.org/10.1111/1365-2664.13383.

Fletcher, M.T., Hungerford, N., Webber, D., Carpinelli de Jesus, M., Zhang, J., Stone, I., Blanchfield, J., Zawawi, N., 2020. Stingless bee honey, a novel source of trehalulose: a biologically active disaccharide with health benefits. Sci. Rep. 10, 12128. https://doi.org/10.1038/s41598-020-68940-0.

Földi, C., Sauermann, S., Dohrmann, R., Mansfeldt, T., 2018. Traffic-related distribution of antimony in roadside soils. Environ. Pollut. 237, 704–712. https://doi.org/10.1016/j.envpol.2017.10.112.

Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570–574. https://doi.org/10.1126/science.1111772.

Formicki, G., Greń, A., Stawarz, R., Zyśk, B., Gał, A., 2013. Metal content in honey, propolis, wax, and bee pollen and implications for metal pollution monitoring. Pol. J. Environ. Stud. 22, 99–106.

Fowler, D., Skiba, U., Nemitz, E., Choubedar, F., Branford, D., Donovan, R., Rowland, P., 2004. Measuring aerosol and heavy metal deposition on urban woodland and grass using inventories of 210Pb and metal concentrations in soil. Water Air Soil Pollut. 4, 483–499. https://doi.org/10.1023/B:WAFO.0000028373.02470.ba.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third ed. Sage, Thousand Oaks ⟨https://socialsciences.mcmaster.ca/jfox/Books/Companion/⟩.

Fritsch, C., Cœurdassier, M., Giraudoux, P., Raoul, F., Douay, F., Rieffel, D., de Vaufleury, A., Scheifler, R., 2011. Spatially explicit analysis of metal transfer to biota: influence of soil contamination and landscape. PLoS One 6, e20682. https://doi.org/10.1371/journal.pone.0020682.

Garibaldi, L.A., Aizen, M.A., Klein, A.M., Cunningham, S.A., Harder, L.D., 2011. Global growth and stability of agricultural yield decrease with pollinator dependence. Proc. Natl. Acad. Sci. U.S.A. 108, 5909–5914. https://doi.org/10.1073/pnas.1012431108.

Georgopoulos, P., Yonone-Lioy, M., Opiekun, R., Lioy, P., 2001. Environmental copper: its dynamics and human exposure issues. J. Toxicol. Environ. Health Part B: Crit. Rev. 4, 341–394. https://doi.org/10.1080/109374001753146207.

Giannini, T.C., Cordeiro, G.D., Freitas, B.M., Saraiva, A.M., Imperatriz-Fonseca, V.L., 2015. The dependence of crops for pollinators and the economic value of pollination in Brazil. J. Econ. Entomol. 108, 849–857. https://doi.org/10.1093/jee/tov093.

Giglio, A., Ammendola, A., Battistella, S., Naccarato, A., Pallavicini, A., Simeon, E., Tagarelli, A., Giulianini, P.G., 2017. Apis mellifera ligustica, Spinola 1806 as bioindicator for detecting environmental contamination: a preliminary study of heavy metal pollution in Trieste, Italy. Environ. Sci. Pollut. Res. Int. 24, 659–665. https://doi.org/10.1007/s11356-016-7862-z.

Gizaw, G., Kim, Y., Moon, K.H., Choi, J.B., Kim, Y.H., Park, J.K., 2020. Effect of environmental heavy metals on the expression of detoxification-related genes in honey bee Apis mellifera. Apidologie 51, 664–674. https://doi.org/10.1007/s13592-020-00751-8.

Goryainova, Z., Vuković, G., Aničić Urošević, M., Vergel, K., Ostrovnaya, T., Frontasyeva, M., Zechmeister, H., 2016. Assessment of vertical element distribution in street canyons using the moss Sphagnum girgensohnii: a case study in Belgrade and Moscow cities. Atmos. Pollut. Res. 7. https://doi.org/10.1016/j.apr.2016.02.013.

GRASS Development Team, 2015. Geographic Resources Analysis Support System (GRASS 6) Programmer's Manual. Open-Source Geospatial Foundation Project. Electronic document: ⟨http://grass.osgeo.org/programming6/⟩.

Grembecka, M., Szefer, P., 2013. Comparative assessment of essential and heavy metals in fruits from different geographical origins. Environ. Monit. Assess. 185, 9139–9160. https://doi.org/10.1007/s10661-013-3242-z.

Gutiérrez, M., Molero, R., Gaju, M., van der Steen, J., Porrini, C., Ruiz, J.A., 2015. Assessment of heavy metal pollution in Córdoba (Spain) by biomonitoring foraging honeybee. Environ. Monit. Assess. 187, 651. https://doi.org/10.1007/s10661-015-4877-8.

Holt, E.A., Miller, S.W., 2011. Bioindicators: using organisms to measure environmental impacts. Nat. Educ. Knowl. 2, 2–8.

Huihui, Z., Xin, L., Zisong, X., Yue, W., Zhiyuan, T., Meijun, A., Yuehui, Z., Wenxu, Z., Nan, X., Guangyu, S., 2020. Toxic effects of heavy metals Pb and Cd on mulberry (Morus alba L.) seedling leaves: photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicol. Environ. Saf. 195, 110469 https://doi.org/10.1016/j.ecoenv.2020.110469.

Hutchinson, T.C., Whitby, L.M., 1977. The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the sudbury smelting region of Canada. Water Air Soil Pollut. 7, 421–438. https://doi.org/10.1007/bf00285542.

Ibe, F.C., Opara, A.I., Ibe, B.O., Adindu, B.C., Ichu, B.C., 2018. Environmental and health implications of trace metal concentrations in street dusts around some electronic repair workshops in Owerri, Southeastern Nigeria. Environ. Monit. Assess. 190, 696. https://doi.org/10.1007/s10661-018-7023-6.

Khosheghbal, M.Z., Esmaeilzadeh, M., 2018. Evaluating of heavy metal pollution in Amir-Kalayeh wetland using geochemical and statistical analyses. Water Sci. Technol. 78, 1276–1286. https://doi.org/10.2166/wst.2018.396.

Kłos, A., Ziembik, Z., Rajfur, M., Dołhańczuk-Śródka, A., Bochenek, Z., Bjerke, J.W., Tømmervik, H., Zagajewski, B., Ziółkowski, D., Jerz, D., Zielińska, M., Krems, P., Godyń, P., Marciniak, M., Świsłowski, P., 2018. Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and North-eastern Poland. Sci. Total Environ. 627, 438–449. https://doi.org/10.1016/j.scitotenv.2018.01.211.

Kodnik, D., Winkler, A., Candotto Carniel, F., Tretiach, M., 2017. Biomagnetic monitoring and element content of lichen transplants in a mixed land use area of NE Italy. Sci. Total Environ. 595, 858–867. https://doi.org/10.1016/j.scitotenv.2017.03.261.

Legendre, P., Legendre, L., 1998. Numerical Ecology, second ed. Elsevier, Amsterdam, p. 852. ISBN 978-0444892508.

Leita, L., Muhlbachova, G., Cesco, S., Barbattini, R., Mondini, C., 1996. Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. Environ. Monit. Assess. 43, 1–9. https://doi.org/10.1007/bf00399566.

Li, C., Li, F., Wu, Z., Cheng, J., 2015. Effects of landscape heterogeneity on the elevated trace metal concentrations in agricultural soils at multiple scales in the Pearl River Delta, South China. Environ. Pollut. 206, 264–274. https://doi.org/10.1016/j.envpol.2015.07.007.

Li, R., Wu, H., Ding, J., Fu, W., Gan, L., Li, Y., 2017. Mercury pollution in vegetables, grains and soils from areas surrounding coal-fired power plants. Sci. Rep. 7, 46545. https://doi.org/10.1038/srep46545.

Li, S., Yang, L., Chen, L., Zhao, F., Sun, L., 2019. Spatial distribution of heavy metal concentrations in peri-urban soils in Eastern China. Environ. Sci. Pollut. Res. Int. 26, 1615–1627. https://doi.org/10.1007/s11356-018-3691-6.

Li, Y., Gou, X., Wang, G., Zhang, Q., Su, Q., Xiao, G., 2008. Heavy metal contamination and source in arid agricultural soil in central Gansu Province, China. J. Environ. Sci. 20, 607–612. https://doi.org/10.1016/S1001-0742(08)62101-4.

Liu, R., Wang, M., Chen, W., Peng, C., 2016. Spatial pattern of heavy metals accumulation risk in urban soils of Beijing and its influencing factors. Environ. Pollut. 210, 174–181. https://doi.org/10.1016/j.envpol.2015.11.044.

Loganathan, P., Hedley, M.J., Grace, N.D., 2008. Pasture soils contaminated with fertilizer-derived cadmium and fluorine: livestock effects. Rev. Environ. Contam. Toxicol. 192, 29–66. https://doi.org/10.1007/978-0-387-71724-1_2.

Luo, L., Ma, Y., Zhang, S., Wei, D., Zhu, Y.G., 2009. An inventory of trace element inputs to agricultural soils in China. J. Environ. Manag. 90, 2524–2530. https://doi.org/10.1016/j.jenvman.2009.01.011.

Ma, M., Du, H., Wang, D., 2019. A new perspective is required to understand the role of forest ecosystems in global mercury cycle: a review. Bull. Environ. Contam. Toxicol. 102, 650–656. https://doi.org/10.1007/s00128-019-02569-2.

Malagodi-Braga, K., Kleinert, A., 2004. Could Tetragonisca angustula Latreille (Apinae, Meliponini) be effective as strawberry pollinator in greenhouses? Aust. J. Agric. Res. 55, 771–773. https://doi.org/10.1071/AR03240.

Manisha, H., Suresh Pandian, E., 2016. Determining the contribution of nearby power plants to deposited foliar dust: a case study of BTPS. Bokaro 71, 485–499. https://doi.org/10.1007/s00244-016-0309-1.

Martello, F., Andriolli, F., Souza, T., Dodonov, P., Ribeiro, M., 2016. Edge and land use effects on dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) in Brazilian cerrado vegetation. J. Insect. Conserv. 20, 957–970. https://doi.org/10.1007/s10841-016-9928-0.

Mazzei, K., 2007. Corredores de fauna na região Cantareira-Mantiqueira: evidencias geográficas. Tese de Doutorado. Universidade de São Paulo. ⟨https://www.teses.usp.br/teses/disponiveis/8/8135/tde-11122007-094508/pt-br.php⟩. ⟨https://doi.org/10.11606/T.8.2007.tde-11122007-094508⟩.

McGarigal, K., 2012. Fragstats: Spatial Pattern Analysis Program for Categorical and Continuous Maps. University of Massachusetts, Amherst, USA. ⟨https://www.umass.edu/landeco/research/fragstats/downloads/fragstats_downloads.html⟩.

Medeiros, H., Martello, F., Almeida, E., Mengual, X., Harper, K., Grandinete, Y., Metzger, J., Righi, C., Ribeiro, M., 2019. Landscape structure shapes the diversity of beneficial insects in coffee producing landscapes. Biol. Conserv. 238, 108193 https://doi.org/10.1016/j.biocon.2019.07.038.

Mihaly Cozmuta, A., Bretan, L., Mihaly Cozmuta, L., Nicula, C., Peter, A., 2012. Lead traceability along soil-melliferous flora-bee family-apiary products chain. J. Environ. Monit. 14, 1622–1630. https://doi.org/10.1039/c2em30084b.

Morgano, M.A., Teixeira Martins, M.C., Rabonato, L.C., Milani, R.F., Yotsuyanagi, K., Rodriguez-Amaya, D.B., 2010. Inorganic contaminants in bee pollen from Southeastern Brazil. J. Agric. Food Chem. 58, 6876–6883. https://doi.org/10.1021/jf100433p.

Moroń, D., Szentgyörgyi, H., Skórka, P., Potts, S.G., Woyciechowski, M., 2014. Survival, reproduction and population growth of the bee pollinator, Osmia rufa (Hymenoptera: Megachilidae), along gradients of heavy metal pollution. Insect. Conserv. Divers. 7, 113–121. https://doi.org/10.1111/icad.12040.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. https://doi.org/10.1038/35002501.

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for plants. Environ. Chem. Lett. 8, 199–216. https://doi.org/10.1007/s10311-010-0297-8.

Newton, A.C., Boscolo, D., Ferreira, P.A., 2018. Impacts of deforestation on plant-pollinator networks assessed using an agent based model. PLoS One 13, e0209406. https://doi.org/10.1371/journal.pone.0209406.

Nikolić, T.V., Kojić, D., Orčić, S., Vukašinović, E.L., Blagojević, D.P., Purać, Jl, 2019. Laboratory bioassays on the response of honey bee (Apis mellifera L.) glutathione S-transferase and acetylcholinesterase to the oral exposure to copper, cadmium, and lead. Environ. Sci. Pollut. Res. Int. 26, 6890–6897. https://doi.org/10.1007/s11356-018-3950-6.

Nowak, D.J., Greenfield, E.J., Hoehn, R.E., Lapoint, E., 2013a. Carbon storage and sequestration by trees in urban and community areas of the United States. Environ. Pollut. 178, 229–236. https://doi.org/10.1016/j.envpol.2013.03.019.

Nowak, D.J., Hirabayashi, S., Bodine, A., Hoehn, R., 2013b. Modeled PM2.5 removal by trees in ten U.S. cities and associated health effects. Environ. Pollut. 178, 395–402. https://doi.org/10.1016/j.envpol.2013.03.050.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, R.B., Simpson, G., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. Vegan: Community Ecology Package R Package Vegan, .vers. 2.2-1.

de Oliveira, R.C., Queiroz, S.C.N., da Luz, C.F.P., Porto, R.S., Rath, S., 2016. Bee pollen as a bioindicator of environmental pesticide contamination. Chemosphere 163, 525–534. https://doi.org/10.1016/j.chemosphere.2016.08.022.

Orsi, R.O., Funari, S.R., Barbattini, R., Giovani, C., Frilli, F., Sforcin, J.M., Bankova, V., 2006. Radionuclides in honeybee propolis (Apis mellifera L.). Bull. Environ. Contam. Toxicol. 76, 637–640. https://doi.org/10.1007/s00128-006-0967-1.

Osyczka, P., Rola, K., 2019. Integrity of lichen cell membranes as an indicator of heavy-metal pollution levels in soil. Ecotoxicol. Environ. Saf. 174, 26–34. https://doi.org/10.1016/j.ecoenv.2019.02.054.

Paniz, F., Pedron, T., Moreira Freire, B., Placido Torres, D., Silva, F., Lemos, B., 2018. Effective procedures for the determination of As, Cd, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Th, Zn, U and rare earth elements in plants and foodstuffs. Anal. Methods 10, 4094–4103. https://doi.org/10.1039/C8AY01295D.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R, 2021. nlme: Linear and Nonlinear Mixed Effects Models R. Package Version, 3, pp. 1–152.〈https://CRAN〉.

Pleijel, H., Karlsson, G.P., Sild, E., Danielsson, H., Skärby, L., Selldén, G., 1996. Exposure of a grass-clover mixture to ozone in open-top chambers—Effects on yield, quality and botanical composition. Agric. Ecosyst. Environ. 59, 55–62. https://doi.org/10.1016/0167-8809(96)01046-8.

Ponte, S., Moore, E.A., Border, C.T., Babbitt, C.W., Tyler, A.C., 2019. Fullerene toxicity in the benthos with implications for freshwater ecosystem services. Sci. Total Environ. 687, 451–459. https://doi.org/10.1016/j.scitotenv.2019.05.362.

Porrini, C., Sabatini, A., Girotti, S., Ghini, S., Mędrzycki, P., Grillenzoni, F., Bortolotti, L., Gattavecchia, E., Celli, G., 2003. Honey bees and bee products as monitors of the environmental contamination. Apiacta 38, 63–70.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007.

Qiu, M., Li, T., Gao, X., Yin, G., Zhou, J., 2020. Effects of urbanization on Cd accumulation in agricultural soils: from the perspective of accessibility gradient. Sci. Total Environ. 701, 134799 https://doi.org/10.1016/j.scitotenv.2019.134799.

Raynor, G.S., Ogden, E.C., Hayes, J.V., 1974. Enhancement of particulate concentrations downwind of vegetative barriers. Agric. Meteorol. 13, 181–188. https://doi.org/10.1016/0002-1571(74)90045-4.

Core Team, R., 2013. R: A Language and Environment for Statistical Computing R. Found. Stat. Comput., Vienna, Austria doi: 10.1890/0012-9658(2002)083[3097:CFHIWS]2.0.CO;2.

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.V.J., Hirota, M.R.M., 2009. The Brazilian Atlantic forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021.

Richardson, J.B., Moore, L., 2020. A tale of three cities: mercury in urban deciduous foliage and soils across land-uses in Poughkeepsie NY, Hartford CT, and Springfield MA USA. Sci. Total Environ. 715, 136869 https://doi.org/10.1016/j.scitotenv.2020.136869.

Roman, A., 2005. The influence of environment on accumulation of toxic elements in honey bees' body. ISAH 2, 423–426.

Sánchez, M., Antolín-Rodríguez, J., Martín-Ramos, P., González-González, A., Bravo Sánchez, C., Martín-Gil, J., 2017. Evolution of mercury content in agricultural soils due to the application of organic and mineral fertilizers. J. Soils Sediments 17, 927–935. https://doi.org/10.1007/s11368-016-1622-z.

Sartorello, R., 2014. Interações em estudos para conservação: conceitos e técnicas para análises geográficas e ecológicas da paisagem. Tese de Doutorado. Universidade de São Paulo. 〈https://www.teses.usp.br/teses/disponiveis/8/8135/tde-24032015–122734/pt-br.php〉 〈https://doi.org/10.11606/T.8.2014.tde-24032015–122734〉.

Sezgin, N., Ozcan, H.K., Demir, G., Nemlioglu, S., Bayat, C., 2004. Determination of heavy metal concentrations in street dusts in Istanbul E-5 highway. Environ. Int. 29, 979–985. https://doi.org/10.1016/s0160-4120(03)00075-8.

Sheehan, P.J., 1984. Effects on individuals and populations. In: Sheehan, P.J., Miller, D. R., Butler, G.C., Bourdeau, Ph (Eds.), Effects of Pollutants at the Ecosystem Level. John Wiley & Sons Ltd, Chichester, p. 443.

Silici, S., Uluozlu, O.D., Tuzen, M., Soylak, M., 2016. Honeybees and honey as monitors for heavy metal contamination near thermal power plants in Mugla, Turkey. Toxicol. Ind. Health 32, 507–516. https://doi.org/10.1177/0748233713503393.

Skaldina, O., Peraniemi, S., Sorvari, J., 2018. Ants and their nests as indicators for industrial heavy metal contamination. Environ. Pollut. 240, 574–581. https://doi.org/10.1016/j.envpol.2018.04.134.

Slaa, J., 2006. Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica. Insectes Sociaux 53, 70–79. https://doi.org/10.1007/s00040-005-0837-6.

Sodango, T.H., Li, X., Sha, J., Bao, Z., 2018. Review of the spatial distribution, source and extent of heavy metal pollution of soil in China: impacts and mitigation approaches. J. Health Pollut. 8, 53–70. https://doi.org/10.5696/2156-9614-8.17.53.

Stecka, H., Jedryczko, D., Welna, M., Pohl, P., 2014. Determination of traces of copper and zinc in honeys by the solid phase extraction pre-concentration followed by the flame atomic absorption spectrometry detection. Environ. Monit. Assess. 186, 6145–6155. https://doi.org/10.1007/s10661-014-3845-z.

Steffan-Dewenter, I., Munzeberg, U., Burger, C., Thier, C., Tscharntke, T., 2002. Scale-dependent effects of landscape context on three pollinators guilds. Ecology 83, 1421–1432. https://doi.org/10.2307/3071954.

Sumita, N.M., Mendes, M.E., Macchione, M., Guimaraes, E.T., de Lichtenfels, A.J., de Lobo, D.J., Saldiva, P.H., Saiki, M., 2003. Tradescantia pallida cv. purpurea boom in the characterization of air pollution by accumulation of trace elements. J. Air Waste Manag. Assoc. 53, 574–579. https://doi.org/10.1080/10473289.2003.10466197.

Sundseth, K., Pacyna, J.M., Pacyna, E.G., Pirrone, N., Thorne, R.J., 2017. Global sources and pathways of mercury in the context of human health. Int. J. Environ. Res. Public Health 14, 105. https://doi.org/10.3390/ijerph14010105.

Tabarelli, M., Aguiar, A., Ribeiro, M., Metzger, J., Peres, C., 2010. Prospects for biodiversity conservation in the Atlantic forest: lessons from aging human-modified landscapes. Biol. Conserv. 143, 2328–2340. https://doi.org/10.1016/j.biocon.2010.02.005.

Takáč, P., Szabová, T., Kozáková, L., Benková, M., 2018. Heavy metals and their bioavailability from soils in the long-term polluted Central Spiš region of SR. Plant Soil Environ. 55, 167–170. https://doi.org/10.17221/21/2009-PSE.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. Exp. Suppl. 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6.

Tenkouano, G.T., Cumming, B.F., Jamieson, H.E., 2019. Geochemical and ecological changes within Moira Lake (Ontario, Canada): a legacy of industrial contamination and remediation. Environ. Pollut. 247, 980–988. https://doi.org/10.1016/j.envpol.2019.01.019.

Tlak Gajger, I., Kosanović, M., Bilandžić, N., Sedak, M., Čalopek, B., 2016. Variations in lead, cadmium, arsenic, and mercury concentrations during honeybee wax processing using casting technology. Arh Hig Rada Toksikol. 67, 223–228. https://doi.org/10.1515/aiht-2016-67-2780.

Tong, S.T., Lam, K.C., 2000. Home sweet home? A case study of household dust contamination in Hong Kong. Sci. Total Environ. 256, 115–123. https://doi.org/10.1016/S0048-9697(00)00471-X.

Toporcak, J., Legath, J., Kul'kova, J., 1992. Levels of mercury in samples of bees and honey from areas with and without industrial contamination. Vet. Med. 37, 405–412.

Tyler, G., 1983. Rubidium - availability and plant uptake in natural soils. Commun. Soil Sci. Plant Anal. 14, 1075–1089. https://doi.org/10.1080/00103628309367433.

Urrutia-Goyes, R., Hernandez, N., Carrillo-Gamboa, O., Nigam, K.D.P., Ornelas-Soto, N., 2018. Street dust from a heavily-populated and industrialized city: evaluation of spatial distribution, origins, pollution, ecological risks and human health repercussions. Ecotoxicol. Environ. Saf. 159, 198–204. https://doi.org/10.1016/j.ecoenv.2018.04.054.

van der Steen, J.J., de Kraker, J., Grotenhuis, T., 2012. Spatial and temporal variation of metal concentrations in adult honeybees (Apis mellifera L.). Environ. Monit. Assess. 184, 4119–4126. https://doi.org/10.1007/s10661-011-2248-7.

van der Steen, J.J., Cornelissen, B., Blacquiere, T., Pijnenburg, J.E., Severijnen, M., 2016. Think regionally, act locally: metals in honeybee workers in the Netherlands (surveillance study 2008). Environ. Monit. Assess. 188, 463. https://doi.org/10.1007/s10661-016-5451-8.

Veleminsky, M., L. P, Stary, P., 1990. Honeybees (Apis mellifera L.) as environmental monitors of heavy metals in Czechoslovakia. Acta Entomol. Bohemoslov. 87, 37–44.

Vossler, F.G., 2018. Are stingless bees a broadly polylectic group? An empirical study of the adjustments required for an improved assessment of pollen diet in bees. In: Vit, P., Pedro, S.R.M., Roubik, D. (Eds.), Pot-Pollen in Stingless Bee Melittology. Springer International Publishing, pp. 17–28. https://doi.org/10.1007/978-3-319-61839-5.

Zarić, N.M., Ilijević, K., Stanisavljević, L., Gržetić, I., 2017. Use of honeybees (Apis mellifera L.) as bioindicators for assessment and source appointment of metal pollution. Environ. Sci. Pollut. Res. Int. 24, 25828–25838. https://doi.org/10.1007/s11356-017-0196-7.

Zarić, N.M., Deljanin, I., Ilijevic, K., Stanisavljevic, L., Ristic, M., Grzetic, I., 2018. Assessment of spatial and temporal variations in trace element concentrations using honeybees (Apis mellifera) as bioindicators. PeerJ 6, e5197. https://doi.org/10.7717/peerj.5197.

Zhang, J., Yang, R., Li, Y.C., Peng, Y., Wen, X., Ni, X., 2020. Distribution, accumulation, and potential risks of heavy metals in soil and tea leaves from geologically different plantations. Ecotoxicol. Environ. Saf. 195, 110475 https://doi.org/10.1016/j.ecoenv.2020.110475.

Zhelyazkova, H., 2012. Honeybees - Bioindicators for environmental quality. Bulg. J. Agric. Sci. 18, 435–442.

Zhou, J., Liang, J., Hu, Y., Zhang, W., Liu, H., You, L., Zhang, W., Gao, M., Zhou, J., 2018. Exposure risk of local residents to copper near the largest flash copper smelter in China. Sci. Total Environ. 630, 453–461. https://doi.org/10.1016/j.scitotenv.2018.02.211.