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Biomonitoring with Honeybees of Heavy Metals and Pesticides in Nature Reserves of the Marche Region (Italy)

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Abstract The aim of this study was to carry out biomonitoring with honeybees (*Apis mellifera* L.) to assess the presence of pesticides and heavy metals (cadmium, chromium, nickel, lead) in all of the ten nature reserves of the Marche Region (central–eastern Italy). The study was carried out during the spring and summer seasons when the honeybees were active, over 3 years (2008–2010). Twenty-two colonies of honeybees bred in hives were used. Samples of live and dead honeybees and of honey were collected from 11 sampling stations from May to October in each year. No pesticide pollution was found. Significant differences in heavy metal concentrations were found among years, months and sites, and in particular situations. The analysis reveals that high heavy-metal concentrations occurred exclusively in live honeybees. For the seasonal averages, the most detected heavy metal was chromium, which exceeded the threshold more often than for the other elements, followed by cadmium and lead; nickel never exceeded the threshold. The data are discussed with an evaluation of the natural and anthropic sources taken from the literature and from local situations that were likely to involve heavy metal pollution.

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Introduction

Biomonitoring can be defined as the use of bio-organisms or bio-materials to obtain information on certain characteristics of the biosphere. The relevant information in biomonitoring is commonly deduced from either changes in the behavior of the monitored organism or from the concentration of specific substances in the tissues of the monitored organism [1]. The interest in bioindicator-based techniques for the detection and evaluation of environmental contaminants has been increasing [2]. With the correct selection of an organism, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, the ease of sampling, and the absence of any necessary expensive technical equipment [1]. Therefore, it is evident that the selection of a suitable organism as a bioindicator represents a critical step in overall biomonitoring activities. The organism can be further selected on the basis of their accumulative and time-integrative behavior [1].

In the literature, biomonitoring species for trace-element air pollution are often selected on the basis of criteria such as specificity [3], accumulation ratios [4], or the well-defined representation of a sampling site [5]. For the biomonitoring of atmospheric pollution, the honeybee (*Apis mellifera* L.) has been the subject of various investigations, and can be considered an "ideal bioindicator," as defined by Stöcker [6]. *A. mellifera* is an insect that is directly affected by the toxicological conditions of its natural environment. It is a good biological indicator, as it is widespread and sensitive to environmental changes. Indeed, honeybees are exposed to numerous pollutants during their foraging activities, their body hair can

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easily retain atmospheric residues, and they can be contaminated via food resources when gathering pollen and nectar from flowers, or through water [7]. Therefore, since the late 1970s, the honeybee has increasingly been used to monitor pesticides [8–15] and environmental pollution by heavy metals [7, 16–27] in territorial and urban surveys.

Pesticides are scattered both in time and space, and depending on the type of chemical compound, their stability, and their affinity for the target organism and surrounding environment, they can be degraded by various environmental factors over greater or lesser periods of time. Honeybees are extremely sensitive to pesticides. Many honeybees that come into direct contact with an insecticide will not have enough strength to return to their hive and will die in the field or during their return flight. Other honeybees with only marginal contact while visiting flowers of a treated species or gathering nectar and pollen from spontaneous species contaminated by pesticide "drift" will eventually die in the hive. In this case, the honeybee acts as a direct indicator and provides us with information on the form of the residues [28]. The number of dead honeybees in front of a hive is therefore the most important variable to be considered for these contaminants [29], and this can vary according to a number of factors: the toxicity (for honeybees) of the active ingredient used [8], the presence and extension of bloom among cultivated or spontaneous plants, the presence of honeybees on the site and at the time of a chemical treatment, the means used to distribute a pesticide, and the presence of wind, among other factors [28].

In the Marche Region, as in other Italian regions, organophosphorus and pyrethroids are very widely used as insecticides for agriculture purposes [23]. Fungicides are usually considered safe for honeybees; however, triazoles can be synergists for pyrethroids, and can thus induce negative side effects in bees [30].

The fundamental aspect that differentiates heavy metals from other pollutants, like pesticides, is their introduction into the territory and their environmental fate. Heavy metals are released in a continuous manner into the environment by various natural and anthropic sources, and as they do not decay and are characterized by latent toxicity, they are continuously present in the environment and enter into the biological cycles [28]. They are predominately transferred as molecules or particulate matter via the atmosphere, mostly on a large scale. The amounts of anthropogenically derived heavy metals have increased continuously since the beginning of the industrial revolution [31]. Generally, they do not cause honeybee mortality, but they can be deposited on the body hairs and taken back to the hive with the pollen, or they might be absorbed together with the nectar of the flowers, or through the honeydew produced by aphids.

Some compounds, such as chromium (Cr) and nickel (Ni), are widely distributed in the environment, as they are released from natural sources and anthropogenic activities, even if, especially for Cr, they originate more from widespread use in various and specific industries [32, 33]. Cadmium (Cd) and lead (Pb) are prominent examples of anthropogenic environmental metal pollutants, and therefore these are considered to be the principle toxic heavy metals [16].

The aim of the present study was to use honeybees as bioindicators for a regional survey of pesticide and heavy metal environmental air pollution. A three-beekeeping-season study was carried out in all of the ten nature reserves of the Marche Region (central–eastern Italy), to determine the presence of these pollutants in foraging honeybees and honey. Cd, Cr, Ni, and Pb were chosen as the representative heavy metals, the levels of which in the atmosphere represent a reliable index of environmental contamination.

Materials and Methods

Sampling Sites

This biomonitoring study was performed over the years 2008, 2009 and 2010, in all of the ten nature reserves of the Marche Region (see Fig. 1): Riserva Naturale di Abbadia di Fiastra (site A: 43°12'02.77"N 13°24'24.34"E, 309 m above sea level (a.s.l.)), Parco del Conero (site B: 43°34'35.75"N 13°32'17.34"E, 67 m a.s.l.), Riserva Naturale Statale Gola del Furlo (site C: 43°38'33.22"N 12°45'13.99"E, 771 m a.s.l.), Parco Naturale della Gola della Rossa e di Frasassi (site D: 43°26'36.69"N 12°57'03.86"E, 339 m a.s.l.), Parco Naturale Monte San Bartolo (site E: 43°56'28.68"N 12°50'14.03"E, 96 m a.s.l.), Parco Naturale dei Monti Sibillini (Site F: 43°016'13.91"N 13°10'59.32"E, 778 m a.s.l.; site G: 42°59'57.96"N 13°06'52.14"E, 975 m a.s.l.), Riserva Naturale Ripa Bianca (site H: 43°32'04.26"N 13°17'28.55"E, 45 m a.s.l.), Parco del Sasso Simone Simoncello (site I: 43°45'55.33"N 12°20'03.21"E, 772 m a.s.l.), Riserva Naturale Regionale Sentina (site J: 42°53'57.91"N 13°54'15.97 E, 0 m a.s.l.), and Riserva Statale Montagna di Torricchio (site K: 42°58'00.07"N 13°02'15.22"E, 1,282 m a.s.l.). Sites C, D, F, G, I, and K are mostly wilder areas, while sites A, B, and E are surrounded by agricultural environments, and sites H and J are surrounded by industrial and urban environments. Each sampling station consisted of two healthy and homogenous hives (Dadant-Blatt type, at ten combs), which were strategically deployed in the nature reserve areas (one sampling station for each area, except for Parco Naturale dei Monti Sibillini, which had two sampling stations), and were constantly checked for sanitary purposes. The monitoring was performed each year from May to October for each sampling site; for each season, dead honeybees were sampled about 20 times (once a week), while live honeybees and honey were sampled five times (once a month).

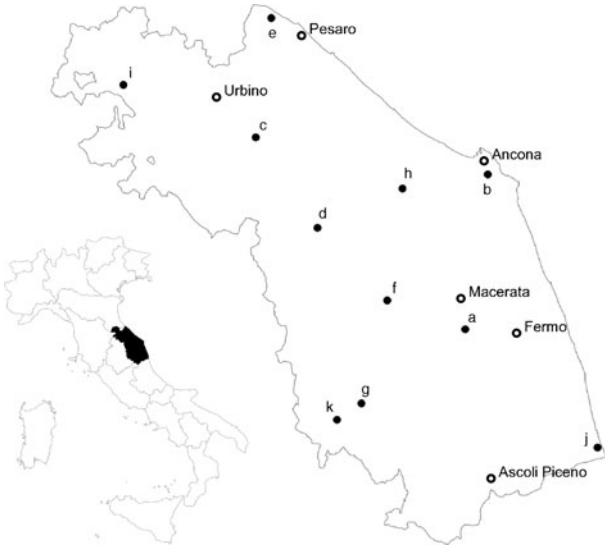

Fig. 1 Map showing Italy and the Marche Region. *Black spots* and *letters* indicate the position of sampling sites: **a** Riserva Naturale di Abbadia di Fiastra, **b** Parco del Conero, **c** Riserva Naturale Statale Gola del Furlo, **d** Parco Naturale della Gola della Rossa e di Frasassi, **e** Parco Naturale Monte San Bartolo, **f**, **g** Parco Naturale dei Monti Sibillini, **h** Riserva Naturale Ripa Bianca, **i** Parco del Sasso Simone Simoncello, **j** Riserva Naturale Regionale Sentina, **k** Riserva Statale Montagna di Torricchio

Biomonitoring of Pesticides

Each hive was equipped with a collection cage for dead honeybees, as an "underbasket" type [34], which was positioned to collect the dead honeybees expelled by the colony. Once a week, the colonies were checked and the number of dead honeybees was recorded. When the mortality rate exceeded the critical threshold (250 honeybees per week per sampling site) [23], pesticide laboratory analyses were performed on the dead honeybee samples.

The determination of pesticides was carried out by gas-chromatography (Carlo Erba, Italy) analysis using specific detection modes: electron capture detector, nitrogen phosphorus detector, and gas chromatography–mass spectrometry. The homogenized samples were mixed with 10 g diatomaceous earth. The mixture was transferred to a cartridge, and the extraction was carried out automatically by accelerated solvent extraction (Dionex, CA, USA), with elution with dichloromethane (Merck, Italy). The extract was then evaporated, without drying, on a rotatory evaporator and taken up in acetone (Merck, Italy). The following chemical classes of pesticides were analyzed: organophosphorus (azinphos-ethyl, azinphos-methyl, chlorpyrifos, chlorpyrifos-methyl, coumaphos, diazinon, dichlorvos, dimethoate, fenamiphos, fenitrothion, fenthion, fonofos, forate, formothion, fosalone, phosphamidon, heptenophos, malathion, methamidophos, methidathion, omethoate, parathion-ethyl, parathion-methyl, pirimiphos-methyl, pyrazophos, pyridaphenthion, quinalphos, tolclofos-methyl, trichlorfon, vamidothion), pyrethroid (acrinathrin, alphamethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenvalerate, flucythrinate, fenpropathrin, fluvalinate, lambda-cyhalothrin, permethrin), and triazoles (bitertanol, bromuconazole, cyproconazole, diclobutrazol, esaconazole, fenbuconazole, flusilazole, flutriafol, myclobutanil, penconazole, prochloraz, propiconazole, tebuconazole, tetraconazole, triadimefon, triadimenol).

Biomonitoring of Heavy Metals

Honey Matrix

Once a month, from May to October for each of the years, samples of honey were collected. Fresh, recently produced, and still unseasoned honey was collected from free cells. In addition, the collection of the honey samples was performed reasonably far from the metallic wire that crossed and supported the honeycombs to avoid any kind of metal contamination. Each sample of honey (70 g) was collected in duplicate from each hive, after which it was stored at 4 °C.

Each 25-g honey sample was mixed with ca. 50 mL distilled water and heated in a water bath at 40 °C for 15 min, to improve and facilitate the handling of the mixture. Then, each sample was cooled and stored at −20 °C. The mineralization was performed for 3 h using a bi-position heating mantle (Falck Instruments, Italy) equipped with reflux condensers (Sigma-Aldrich, Italy): each 5-mL sample was diluted with 10 mL 65 % aqueous solution of nitric acid (Merck, Italy) and 3 mL 30 % aqueous solution of hydrogen peroxide (Merck, Italy). Each sample was then cooled, transferred to a 50-mL volumetric flask, taken to 50 mL with bi-distilled water, and analyzed by inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (Horiba, France) (modified from [35]). Standard solutions consisted of 1.0 g/L nitric acid and 5 % of each element (Cd, Cr, Ni, Pb), at the highest purity available (Merck, Italy). Prior to use, all glassware were treated with a solution of 5 % nitric acid overnight, to avoid any contamination, then rinsed with ultrapure water and dried. Dilute solutions were prepared from standard solutions by dilution with 5 % nitric acid, as appropriate for each element, and then mixed at five concentration levels, which were used for the construction of calibration curves. Ultrapure water was used as the blank.

Live Foraging Honeybee Matrix

Once a month, from May to October of each year, about 100 honeybees were collected from each hive using a modified hand vacuum (Philips, The Netherlands), and placed in sterile, plastic bags. The forager honeybees were sacrificed in the laboratory by freezing at ca. −20 °C, lyophilized at 40 °C, unified by grinding,

and carefully mixed [modified from 36]. Samples of ca. 1 g [36] were mineralized by acid digestion and analyzed by ICP-AES, as described for honey matrix.

Data Analysis

Data from the pesticide biomonitoring were not included in the statistical analysis due to the negative results. The environmental risk thresholds used in this study were defined by Porrini et al. (modified from [23]) on the basis of previous literature and their own experimental data. The mean threshold values were: for the honeybee matrix, Cd, 0.10 mg/kg; Cr, 0.12 mg/kg; Ni, 0.30 mg/kg; and Pb, 0.70 mg/kg; and for the honey matrix, Cd, 0.01 mg/kg; Cr, 0.02 mg/kg; Ni, 0.20 mg/kg; and Pb, 0.05 mg/kg.

The data were log-transformed to meet the assumptions of normality, and analyzed by two-way ANOVA, followed by Tukey tests ($p<0.05$), to reveal any differences among years, months, and sites. The threshold overrun frequencies (percent) were calculated for each heavy metal in the live honeybee and honey matrices.

Results

Biomonitoring of Pesticides

During the beekeeping seasons of 2008, 2009 and 2010, the mortality rate of the foraging honeybees exceeded the critical threshold only twice: for site E on June 28, 2008 (305 dead honeybees) and site J on May 15, 2009 (366 dead honeybees). However, laboratory analyses carried out in the collected samples of the dead honeybees did not show the presence of any of the pesticides.

Biomonitoring of Heavy Metals

The heavy metals exceeded their respective thresholds in different periods, according to the sites. The monthly averages for Cd in the live honeybee matrix exceeded the threshold in 11.6 % (23/198) of cases (Fig. 2a). The monthly averages for Cr in the live honeybee matrix exceeded the threshold in 19.7 % (39/198) of cases (Fig. 2b), while in the honey matrix, this occurred in 11.1 % (22/198) of cases (Fig. 3a). The monthly averages for Ni in the live honeybee matrix exceeded the threshold in 5.1 % (10/198) of cases (Fig. 2c), while in the honey matrix this occurred in 1.5 % (3/198) of cases (Fig. 3b). The monthly averages of Pb in the live honeybee matrix exceeded the threshold in 4.6 % (9/198) of cases (Fig. 2d).

The analysis of the seasonal averages for Cd (Table 1, Online Resources) showed values that exceeded the threshold in the live honeybee matrix for site C in 2008 (+0.05 mg/kg), and site F (+0.01 mg/kg) and site I (+0.05 mg/kg) in 2009. The seasonal averages for Cr (Table 2, Online Resources) showed excess values in the live honeybee matrix for site B in 2009 (+ 0.01 mg/kg), site C in 2008 (+0.06 mg/kg) and 2010 (+0.03- mg/kg), site D in 2010 (+0.03 mg/kg), site F in 2008 (+ 0.01 mg/kg) and 2010 (+0.02 mg/kg), site I in 2008 (+0.48- mg/kg), and site K in 2008 (+0.017 mg/kg). The seasonal averages for Pb (Table 4, Online Resources) showed excess values for site A in 2008 (+0.27 mg/kg).

In comparing the average site values for the heavy metals in the live honeybee matrix within the years, there were significant differences among the sites in 2008 for Cd ($F=2.625$, $p<0.05$) (Table 1, Online Resources), in 2009 for Ni ($F=2.744$, $p<0.01$) (Table 3, Online Resources) and Pb ($F=7.937$, $p<0.001$) (Table 4, Online Resources), and in 2010 for Cd ($F=3.726$, $p<0.01$) (Table 1, Online Resources). The only significant differences among the sites in the honey matrix were seen for Ni in 2009 ($F=4.528$, $p<0.001$) and 2010 ($F=3.921$, $p<0.001$) (Table 3, Online Resources).

By comparing the average monthly values of the heavy metals within the years, significant differences among the months were seen in the live honeybee matrix in 2008 for Cd ($F=2.799$, $p<0.05$) (Table 1, Online Resources) and Cr ($F=4.678$, $p<0.01$) (Table 2, Online Resources), and in 2009 for Cd ($F=2.429$, $p<0.05$) (Table 1, Online Resources) and Cr ($F=4.169$, $p<0.01$) (Table 2, Online Resources). In the honey matrix, this was only seen in 2010 for Ni ($F=5.150$, $p<0.01$) (Table 3, Online Resources).

By comparing the mean seasonal values of the heavy metals within each site, significant differences were observed among the years (Table 5, Online Resources). In the live honeybee matrix, this was seen for site B for Cr ($F=4.490$, $p<0.05$) and Ni ($F=9.719$, $p<0.01$), for site E for Cr ($F=6.509$, $p<0.01$) and Pb ($F=13.636$, $p<0.001$), for site F for Cd ($F=5.134$, $p<0.05$) and Ni ($F=15.362$, $p<0.001$), for site G for Ni ($F=29.319$, $p<0.001$), and for site H for Cr ($F=3.905$, $p<0.5$). For the honey matrix, this was seen only for Ni, for site C ($F=9.207$, $p<0.01$), for site D ($F=17.839$, $p<0.001$), for site F ($F=16.176$, $p<0.001$), and for site I ($F=24.490$, $p<0.001$).

Discussion

The weekly screenings conducted to determine the mortality of the two hives in each of the biomonitoring sites showed threshold overruns for only two dates, although these analyses did not reveal the presence of pesticides. The high mortality was probably associated with the confirmed swarming. Natural areas in the Marche Region have different types of flora over different extents. As pesticides were not detected, this might indicate that the sites are completely surrounded by wild environments, although this assumes different values

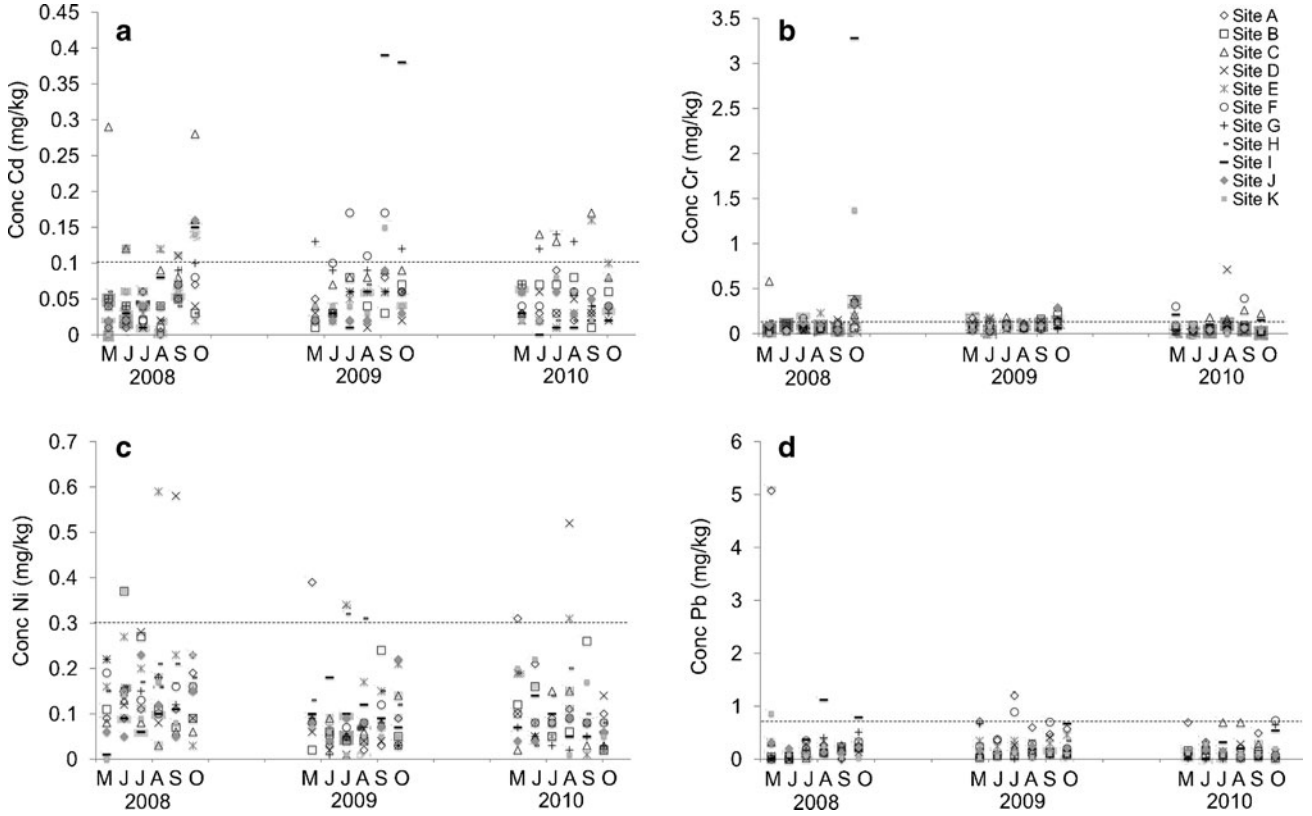

Fig. 2 Scatter plots showing the concentrations of Cd (**a**), Cr (**b**), Ni (**c**), and Pb (**d**) in the honeybee matrix, during May (*M*), June (*J*), July (*J*), August (*A*), September (*S*), and October (*O*) of 2008, 2009 and 2010, as indicated. The *dashed lines* show heavy metal threshold values: Cd, 0.10 mg/kg; Cr, 0.12 mg/kg; Ni, 0.30 mg/kg; Pb, 0.70 mg/kg. The *different symbols* represent the different sampling sites

for areas in which the biomonitoring site is surrounded by anthropogenic and agricultural activities. Therefore, these data show that during the 3 years of investigation, there was no contamination due to non-rational use of pesticides in any of these Nature Reserves of the Marche Region. This is in line with the statistical analyses conducted from 2001 to 2011 by the Italian National Institute of Statistics, which showed that the Marche Region is one of the regions with the lowest distribution of pesticides per hectare [37].

The analysis of the seasonal averages showed higher values of heavy metals exclusively in the live honeybees. The most prevalent heavy metal was Cr, which exceeded the threshold more often than the other heavy metals, while the seasonal averages for Ni never exceeded the threshold. The

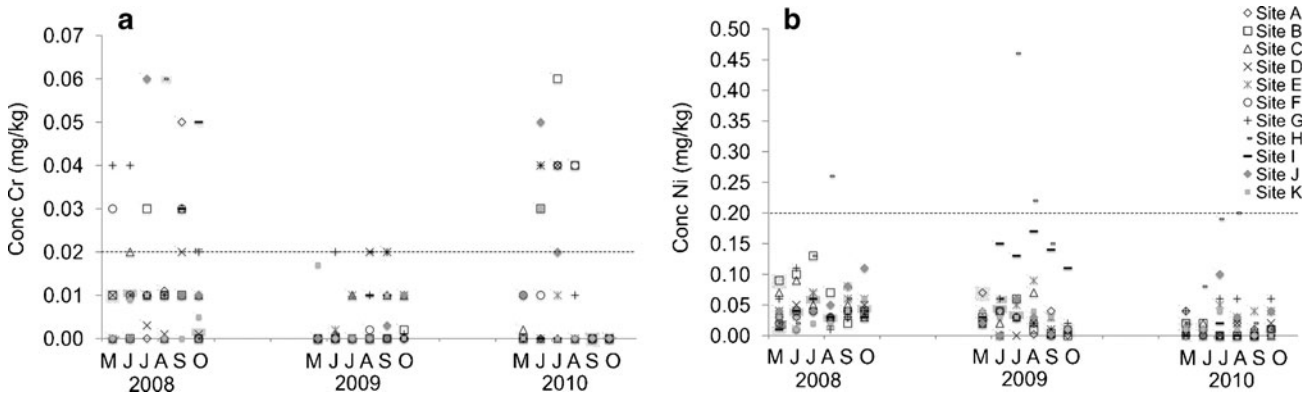

Fig. 3 Scatter plots showing the concentrations of Cr (**a**) and Ni (**b**) in the honey matrix, during May (*M*), June (*J*), July (*J*), August (*A*), September (*S*), and October (*O*) of 2008, 2009 and 2010, as indicated. The *dashed lines* show heavy metal threshold values: Cr, 0.02 mg/kg; Ni, 0.20 mg/kg. Cd and Pb were not detected in the honey matrix. The *different symbols* represent the different sampling sites

overrun occurred mostly in the wilder mountain sites, as if their altitude acted as a natural barrier for accumulation. During the year in which the heavy metal thresholds were more often exceeded, the weather conditions were very dry (data not shown), which might have caused the failure of the process of heavy metal leaching out of the flowers. However, it is important to note that in all of these cases, the threshold was never exceeded in the corresponding honey matrix. This missing correspondence can be explained by the biology of honeybees: after 3 weeks, the workers become foragers, and they gather pollen, nectar, honeydew, and water for the colony until their death [38], and as these honeybees are the only ones to get out of the hive, they are the ones involved in the accumulation of heavy metals from the environment. Therefore, this might represent a point event of environmental pollution, which was enough to pollute the foragers only in a given period, but not enough to be accumulated in the honey.

Accordingly, critical situations were observed for sites where the thresholds were exceeded in particular months in both matrices, so in the live honeybees and in the honey, this would confirm a continuous polluting event. These situations occurred for only two sites: during the summer for Ni and in early autumn for Cr.

The most common of these heavy metals in the Marche Region was definitely Cr, which showed the highest threshold overruns. Kotaś and Stasicka [33] and Seigneur and Constantinou [39] demonstrated that 30 % to 40 % of the Cr in the atmosphere originates from natural sources, such as the weathering of rock constituents, wet precipitation, dry fallout from the atmosphere, and runoff from the terrestrial systems; the remaining 60 % to 70 % comes from anthropogenic sources. Indeed, Cr is a significant worldwide problem [40], with interest in it originating from widespread use of Cr in various industries, such as for chrome colors and dyes, cement manufacture, and wood preservatives [33, 41]. Therefore, large quantities of Cr compounds are discharged as liquid, solid, and gaseous pollutants into the environment and will ultimately have significant adverse biological and ecological effects. The amount of Cr at any particular time and location will thus depend mostly on the intensity of the industrial processes in the proximity of the sources, the amount of Cr released, and the meteorological factors [33]. In the present study, it can be noted that the Cr threshold was exceeded during the month of October for almost all of the sites. This situation appears to be caused by the regional industrial activities and the weather conditions: the higher frequency of excess over the thresholds might have been driven by the combination of anthropogenic activities and the lack of rainfall recorded in the period before the sampling. In support of this thesis, the Cr threshold was not exceeded during the year in which the rainfall during the late summer was higher (data not shown).

During these 3 years of the survey, Cd exceeded the threshold only in the live honeybee matrix. Indeed, from the literature it appears that Cd is deposited at its highest levels in the hemolymph of the bees [27]. Cd is naturally present in the soil and in sediments [42], but it mainly originates from domestic and metal industry combustion processes, whereby it is transported from the soil to plants, consequently contaminating the nectar and the honey [16, 31]. Cd is one of the most dangerous heavy metals due to its high mobility and the small concentrations at which its effects on plants are seen [43]. The presence of this contaminant in the live honeybee matrix can be explained according to Yaaqub et al. [44], who showed that 33 % to 72 % of the local Cd is supplied from the air, and according to Harrison and Williams [45], who explained that airborne Cd is transferred predominately by large-scale atmospheric transport. Moreover, the absence of Cd in the honey matrix might indicate low concentrations of this element, which might not be sufficient to contaminate the nectar of the foraged plants.

Also in the case of Pb, the threshold was exceeded only in the live honeybee matrix. As suggested by Lambert et al. [7], as honeybees appear to be more sensitive to Pb contamination, this could be linked to exposure to Pb during their flight, so the peaks of this contamination in the bees should reflect very occasional episodes of atmospheric contamination. Perugini et al. [22] reported similar results with a significant difference in Pb concentration in honeybees through comparing sites located in urban areas and sites located in nature reserves. In their case, the highest mean concentrations were detected in honeybees collected from hives for which the surroundings were characterized by intense air traffic and intense motor vehicle circulation. This hypothesis is not directly supported by the present study because the nature reserves that showed the higher values of Pb were mostly surrounded by a wilder environment. However, this might also indicate drift, and the accumulation of Pb from the polluted atmosphere of the nearest urbanized areas.

In the literature, Ni is considered to be one of the many trace metals that are widely distributed in the environment, as it is released from both natural sources and anthropogenic activity [32]. Ni has many industrial and commercial uses, and it has been increasing in worldwide importance with the application of new technologies [32, 46]. Although Ni is omnipresent and is vital for the function of many organisms, its high concentrations in some areas from both anthropogenic release and naturally varying levels might be toxic to living organisms [32]. Despite this, during these 3 years of survey, Ni exceeded the threshold only a few times in the live honeybees and honey. This can be explained as it finds its way into the ambient air as a result of the combustion of coal, diesel oil and fuel oil, the incineration of waste and sewage, and from miscellaneous sources [47, 48], and these are activities that are slightly evident in the Marche Region.

These data for the heavy metal content of the honey also support the previous data from Leita et al. [21], Fakhim-Zadeh and Lodenius [20], and Porrini et al. [23], in which there was again relatively low contamination of honey. This is probably due to "filtering" by the honeybees, as honey has a considerably lower heavy metal content than live honeybees.

The possibility of using honeybees as bioindicators of environmental pollution takes advantage of the large areas that they can cover where they live, and the detection of the presence of heavy metals that are harmless to them. While mechanical instruments give more precise values, honeybees provide data over the full area that they cover during foraging. According to Wolterbeek [1], the relative ease of sampling, the absence of any need for complicated and expensive technical equipment, and the accumulative and time-integrative behavior of the honeybees as monitor organism means that such biomonitoring of atmospheric trace elements will be continued for the foreseeable future, especially with larger-scale surveys. Therefore, as suggested by Leita et al. [21], a network of hives can supply data for the constant monitoring of heavy metal emissions from pinpoint sources.

In the Marche groundwater regional network, about 24 % of the monitoring points showed contamination by heavy metals, including Cr, Cd and Pb [49]. Water quality data analyses of several rivers in the Marche Region, which are located in areas with neighboring industrial activities, have revealed the presence of a mixture of low levels of heavy metals [50]. Moreover, sediment analyses from the Cesano, Metauro, Tavollo, and Musone Rivers showed high levels of Cr and/or Ni [50]. Senesi et al. [51] reported high values of Pb in sandy clay loam soil sampled in the Fano municipality. A biomonitoring survey of lichen carried out over a large area characterized by high impact of industrial and urban sources of air pollution in Ancona Province reported high levels of Cd, Cr, Pb, and Ni in the atmosphere [52].

However, the survey carried out in the present study has revealed good overall air environmental quality of the biomonitoring sites with regard to pesticides and heavy metals, even if for the heavy metals there was the presence of some potentially critical situations with Cr.

Therefore, the collected data over these 3 years of the present study provide a first examination of the presence and levels of these heavy metals in the air of these natural environments throughout the whole of the Marche Region. Such a wealth of data can be considered as a basic reference point for future investigations of environmental monitoring, and we expect that this study will be the start of further investigations to promote our understanding of the sources of environmental pollution in the Marche Region.

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