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Identifying Sources of Environmental Contamination in European Honey Bees (*Apis mellifera*) Using Trace Elements and Lead Isotopic Compositions

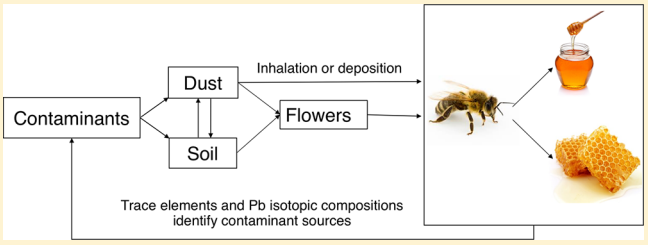
Xiaoteng Zhou,[†] Mark Patrick Taylor,[*,†,‡] Peter J. Davies,[†,‡] and Shiva Prasad[§]

[†]Department of Environmental Sciences, Faculty of Science and Engineering and [‡]Energy and Environmental Contaminants Research Centre, Macquarie University, North Ryde, Sydney, NSW 2109, Australia

[§]Analytical Service Branch, National Measurement Institute, Department of Industry, Innovation and Science, Australian Government, Riverside Corporate Park, North Ryde, Sydney, NSW 2113, Australia

Supporting Information

ABSTRACT: Trace element concentrations (As, Mn, Pb, and Zn) and Pb isotopic compositions were analyzed in honey bees, wax, and honey along with co-located soil and dust samples from Sydney metropolitan and Broken Hill, Australia. Compared with the other trace elements, Pearson correlations show that Pb concentrations in soil and dust had the strongest relationship to corresponding values in honey bees and their products. Dust Pb was not only highly correlated to corresponding soil values ($r = 0.806$, $p = 0.005$), it was the strongest predictor of Pb concentrations in honey bees, wax, and honey ($p = 0.001$, 0.007, 0.017, respectively). Lead isotopic compositions ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) showed that honey bees and their products from Broken Hill were nearly identical (95−98%) to the composition of the local ore body. Samples of honey bees and their products collected from background sites adjacent to national parks in Sydney had Pb isotopic compositions ($^{206}Pb/^{207}Pb$ = 1.138−1.159, $^{208}Pb/^{207}Pb$ = 2.417−2.435) corresponding to local geogenic values ($^{206}Pb/^{207}Pb$ = 1.123−1.176, $^{208}Pb/^{207}Pb$ = 2.413−2.500). By contrast, honey bees and their products from Sydney metropolitan ($^{206}Pb/^{207}Pb$ = 1.081−1.126, $^{208}Pb/^{207}Pb$ = 2.352−2.408) were similar to aerosols measured during the period of leaded petrol use ($^{206}Pb/^{207}Pb$ = 1.067−1.148, $^{208}Pb/^{207}Pb$ = 2.341−2.410). These measurements show Pb concentrations and its isotopic compositions of honey bees, and their products can be used to trace both legacy and contemporary environmental contamination, particularly where sources are well documented. Moreover, this study demonstrates that legacy Pb emissions continue to be remobilized in dust, contaminating both food and ecological systems.



INTRODUCTION

Urban agriculture is growing rapidly in popularity.[1,2] As part of the urban agricultural revolution, European honey bees (*Apis mellifera*) are increasingly being kept in cities,[3] which are known to be polluted by a range of environmental contaminants.[4] Honey bees are a critical part of the agricultural system and pollinate approximately two-thirds of crops that provide 90% of global food.[5] The global economic value of insect pollination services, which are dominated by honey bees, was estimated to be worth €153 billion (US$187 billion) in 2005,[6] with recent estimates indicating that annual honey bee pollination is worth >USD$15 billion in the USA[7] and around AUD$4−6 billion in Australia.[8]

In a single beehive of *Apis mellifera*, there is one queen bee, and up to a few thousand drones, and tens of thousands of worker honey bees.[9] Drones are relatively inactive, stay in the beehive most of the time, and their only role is to mate with the queen.[10] By contrast, worker honey bees spend a significant amount of their adult life outside of the hive foraging over an area of 7 km^2 or more for nectar, pollen, and water.[11,12] Consequently, honey bees have been considered as a potential sentinel species for monitoring trace element contamination in the environment.[12,13]

Trace elements are ubiquitous in the environment, occurring either from natural processes (forest fires, volcanic emissions, sea spray, and biogenic sources) or anthropogenic activities such as agrochemicals, industry, mining and mineral processing, and traffic emissions.[14−16] The combustion of coal (e.g., Hg, Mn, Se, Sn) and oil (e.g., Ni, V) are major sources of trace metal emissions to the environment along with those from nonferrous metal production (e.g., As, Cd, Cu, Pb, Zn).[16] Emissions of Pb along with other co-contaminants from vehicle brakes and tires (As, Cd, Cr, Ni) was dominated by the combustion of leaded petrol, which emitted millions of tonnes to the atmosphere.[17−20] The accumulation of certain trace elements in the environment can have a detrimental impact on ecosystems and living organisms where there are exposure pathways.[21]

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Ingestion and inhalation of elevated concentrations of trace elements including As, Pb, Mn, and Zn have been associated with a variety human health problems arising from chronic or acute exposures.[22] In particular, As and Pb are considered toxic, even in minute amounts.[23] Although the global withdrawal of leaded petrol is almost complete[24] and its significance as a global atmospheric contaminant has declined rapidly in the last 20 years,[25] it continues to remain an environmental health challenge. Not only does Pb persist in the environment, but it has contaminated urban environments leading to adverse human health outcomes even from low exposures.[26,27]

The use of standard methods to characterize large-area spatial variations of trace elements requires evenly distributed sample collection sites, which are costly.[28] In order to overcome the cost and challenge of setting up a well-distributed sampling regime, the use of indicator species such as honey bees has been suggested as a more efficient way of gathering data to monitor environmental quality and the concentrations of contaminants.[13,29,30] On the basis of worker honey bees' extensive foraging behavior, it is more likely that they have greater potential as a environmental bioindicator[31,32] than drones from the same hive. During their short life cycle in summer (2−4 weeks), the worker honey bee completes thousands of interactions with soil, air, water, and vegetation.[33] During foraging, worker honey bees make contact and interact with contaminant particles derived from soil and dust, which adhere to their body parts and hairs.[13] Adhered contaminants are returned to the beehive, influencing the trace element composition of its honey and wax.[4] Environmental contaminants can also accumulate in and on the bodies of worker honey bees.[13]

The use of honey bees and their products as bioindicators for environmental contamination was first attempted by Svoboda in 1962 using strontium (^{90}Sr).[34] A number of recent international studies have examined the use of honey bees and their products as biomarkers to detect environmental contamination by trace elements.[13,35−39] However, the utility of honey bees or their products for use as a reliable bioindicator has had mixed success in previous studies,[40−42] often because of the absence of quantitative assessment of the relationship between honey bees and co-located soils[43] and dusts.[44] This is the first Australian study to measure and analyze the relationships between trace element concentrations (As, Mn, Pb, Zn) in European honey bees, honey, and wax and corresponding temporal soil and dust samples. Samples for this study were collected from Australia's largest city, the Sydney metropolitan area, and the >130-year old Pb−Zn−Ag mining city of Broken Hill, New South Wales, approximately 1160 km west of Sydney (Figure 1). Unlike other investigations of trace elements in honey bees and their products, this study uses Pb isotopic compositions to discriminate potential sources of contamination such as former petrol Pb and mining emissions compared with background geogenic materials.

MATERIALS AND METHODS

Study Area. Sample locations in Sydney included sites close to national parks (Galston and Gordon), low to medium density coastal residential suburbs (Coogee and Randwick), high density city areas of Sydney (central business district (CBD), Marrickville, Newtown, Surry Hills), and a mixed land use suburb containing light industrial, commercial, and residential uses (Mascot). The inner city areas of Sydney have been most affected by former lead petrol emissions, with diminishing effects away from the CBD.[28,45] Mascot is adjacent to Sydney Airport, which is the oldest and busiest continually operating commercial airport in Australia. Samples from the Pb−Zn−Ag mining city of Broken Hill were included in the study because it is still subject to ongoing atmospheric contamination that significantly impacts trace element concentrations in the immediate local environment (especially Pb).[46−48] Contamination from Broken Hill Pb and other Australian ores with a similar Pb isotopic signature (e.g., Mount Isa ore) from leaded petrol and mine-related emissions can be identified in global environmental archives,[49−52] with it still being persistent in contemporary Antarctic ice[25] and ocean waters.[53]

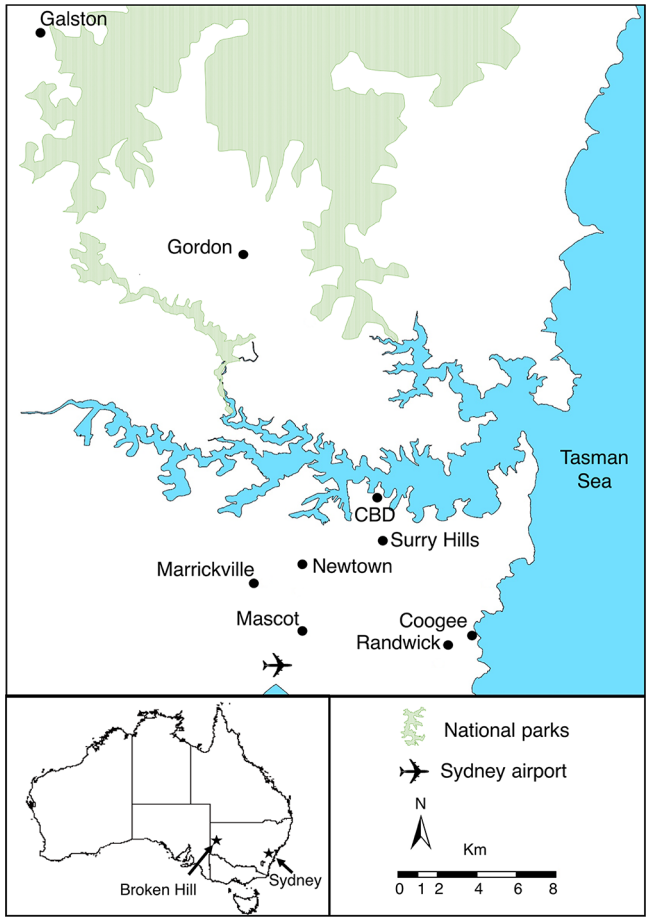

Figure 1. Location of beehive sites sampled in Sydney metropolitan area (n = 9) and Broken Hill (n = 1). Sample locations are adjacent to the following land uses: national park (Galston, Gordon), coastal residential suburbs (Coogee, Randwick), high density inner city (Sydney CBD, Surry Hills, and Newtown), residential−industrial (Marrickville), airport and light industrial, commercial, and residential (Mascot) and Pb−Zn−Ag mining (Broken Hill).

Sample Collection. Ten European honey bee hives (*Apis mellifera*) (nine across Sydney metropolitan and one from Broken Hill) were sampled in the warmer Australian months between spring and late summer (November 2015 to April 2016), the most intensive foraging period for honey bees. Thirty live worker honey bees were collected at monthly intervals by use of a bug vacuum at the entrance of each beehive. In addition, 60 live drones were collected from the beehives at Coogee (n = 30) and Randwick (n = 30) in November 2015. Thirty dead worker honey bees were collected on two separate occasions (November 2015 and April 2016) from the area in front of the beehives at the Surry Hills city site.

Raw, unprocessed samples of honey (n = 17) and wax (n = 16) produced during the study period were collected from all

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10 beehives (Figure 1). These were supplemented with additional honey samples from Marrickville (two samples) and Paddington (one sample), which is an older residential area some 3.3 km southeast of the CBD.

Trace metal clean techniques were employed for sample collection and processing using established procedures as detailed below. Dust deposition samples were collected using published methods.[47] Dust sample collection equipment was constructed in accordance with the Australian Standard 3580.10.1−2003[54] and consisted of a 150 mm diameter glass funnel inserted inside a 2.75 L glass bottle, secured in a plastic bucket affixed to a ∼ 2 m high tripod. Funnels were rinsed with Milli-Q (Milli-Q) water during each replacement. Sample bottles were sealed in the field and transported to the National Measurement Institute (NMI), North Ryde, Sydney, for analysis. Dust samples (n = 41) were collected monthly at the same time as honey bee collection over the sampling period. Established methods[55] were used to collect surface soils (0−2 cm) (n = 44) around each beehive at the beginning of the study in November 2015. Soil samples were oven-dried at 40 °C for 48 h and sieved to <2 mm using a stainless steel mesh prior to trace element analysis at the NMI.

Sample and Data Analysis. Samples of bees, honey, wax, soil, and dust were analyzed at the NMI, using National Association of Testing Authorities (NATA) accredited in-house reference methods for food, soil and dust. Raw, unprocessed honey bees and honey samples were used for analysis. Wax samples were rinsed with Milli-Q water until all honey residues were removed and then oven-dried at 60 °C. One gram of honey bees, honey, and wax was digested with 3 mL of HNO_3 before heating at 100 °C for 2 h.

Soil samples were digested by adding HCl and HNO_3 (1:1 respectively, 6 mL) followed by heating at 100 °C for 1 h. After cooling, 10 mL of Milli-Q water was added and the sample reheated to 100 °C for another 1 h. Dust deposition samples were collected on glass microfibre filters (Whatman 934-AH, 47 mm diameter), and after weighing, digested using HCl and HNO_3 (1:3, respectively, 8 mL) for 2 h. Each digested sample of honey bees, honey, wax, soil, and dust was topped up to 40 mL with Milli-Q. Samples were diluted prior to analysis for their As, Mn, Pb, and Zn concentrations on an inductively coupled plasma mass spectrometer (Agilent 7900 equipped with an ISIS sample introduction system). Each sample batch (n = 20) contained a laboratory reagent blank and duplicate, blank spike, blank matrix, duplicate sample, and matrix spikes.

Procedural blanks were below NMI's Limit of Reporting (LOR) of 10 μg/kg for As, Mn, Pb, and Zn in honey bees, honey, and wax. Dust sample blanks were < LOR of 0.1 mg/kg and 0.05 mg/kg for As and Zn, Pb and Mn, respectively. Procedural blanks for the soil samples were < LOR of 0.05 mg/kg for As and 0.01 mg/kg for Pb, Mn, and Zn. The NMI's internal reference materials AGAL-10 (Hawkesbury River Sediment, n = 4) and AGAL-12 (biosoil, n = 4) were processed with soil and dust samples. Mean recovery rates of As, Mn, Pb, and Zn in AGAL-10 and AGAL-12 were 101% and 108%, respectively. Recovery rates for As, Mn, Pb, and Zn ranged between 96−99% for soil and 98−100% for dust. Matrix spike recovery rates for As, Mn, Pb, and Zn for honey bees, honey and wax were 99−106%. Analytical uncertainties for all elements were 14−24%.

Sample heterogeneity was assessed by replicate analysis (n = 4) of the different study sample matrices along with NMI's internal reference materials AGAL-10 and AGAL-12. The relative standard deviations (RSDs) for all study matrices (honey bees, honey, wax, dust. and soil) were less than 5%. The exceptions were As in honey bees and wax at 7.5% and 6.4%, respectively, and Pb concentration in honey bees, which was 22.7% in samples from Sydney CBD. The elevated sample RSD in a bulk 1 g sample of honey bees prompted a more detailed investigation of sample heterogeneity involving analysis of Pb concentrations in individual live worker honey bees from the Sydney CBD (n = 12) and Surry Hills sites (n = 14). These analyses returned Pb concentration RSDs of 26% and 31.5%, respectively. The implications of this are considered below. The RSDs for the NMI's internal reference materials ranged between 0.3% and 1.4%.

Samples of honey bees (n = 26), honey (n = 6), wax (n = 6), soil (n = 42), and dust (n = 35) were subjected to Pb isotopic composition analysis ($^{204}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$) after sample volumes were optimized on the basis of their Pb concentrations. The certified values of $^{204}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, and $^{208}Pb/^{207}Pb$ for National Institute of Standards and Technology (NIST) SRM981 (natural Pb isotope composition standard) are 0.0646 ± 0.000047, 1.0933 ± 0.00039, and 2.3704 ± 0.0012, respectively. The measured values (n = 164) were 0.066 ± 0.001, 1.103 ± 0.005, and 2.389 ± 0.010. The overall limits of error for NIST SRM981 certified values and measured values are based on 95% confidence limits for the mean of the ratios measured. The NIST SRM981 was used to correct for mass discrimination. Analytical uncertainties for Pb isotopic compositions (expressed for the NIST SRM981) were $^{204}Pb/^{207}Pb$ = 0.065 ± 0.0005, $^{206}Pb/^{207}Pb$ = 1.093 ± 0.005, $^{208}Pb/^{207}Pb$ = 2.370 ± 0.01, respectively. The mean RSDs for NIST981 $^{204}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$ were 0.55%, 0.23%, 0.22%, respectively. The mean RSDs for sample analysis $^{204}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$ were 0.20%, 0.13%, and 0.23%.

Data were analyzed using SPSS 22.0 statistics software for arithmetic mean, standard deviation, and minimum and maximum values, and Minitab Express v 1.5.1 was used for generating linear regressions and tests of significance (Pearson correlations and t tests). Concentrations of As, Mn, Pb, and Zn in honey bees, honey, and wax with <LOR (10 μg/kg) are considered as 5 μg/kg for statistical analysis. Trace element concentrations in various beehive products and environmental samples of soil and dust were non-normally distributed and were $\log_{10}$ transformed for correlation analysis. Pearson correlation coefficients and two-tailed tests of significance were applied to assess the relationships between beehive products (honey bees, honey, wax) and the environment (soil, dust) across the sampling sites. Two sample t tests were used to compare concentrations of As, Mn, Pb, and Zn in worker honey bees and drones, live worker honey bees, and dead worker honey bees.

RESULTS

Trace Elements in Worker and Drone Honey Bees. Given that worker and drone honey bees have different life cycles and roles in a hive, with the latter being less active in the outside environment, their trace element concentrations were compared to ascertain if there were discernible differences in exposures. Higher, but nonsignificant, levels of As, Mn, Pb, and Zn were found in worker honey bees compared to drones (Figure 2) (p = 0.284−0.404, two-sample t tests). Concentrations of As and Zn in worker honey bees were found to be double that in drones, with Pb being four and Mn eight times greater (Table S1). Dead worker honey bees contained higher

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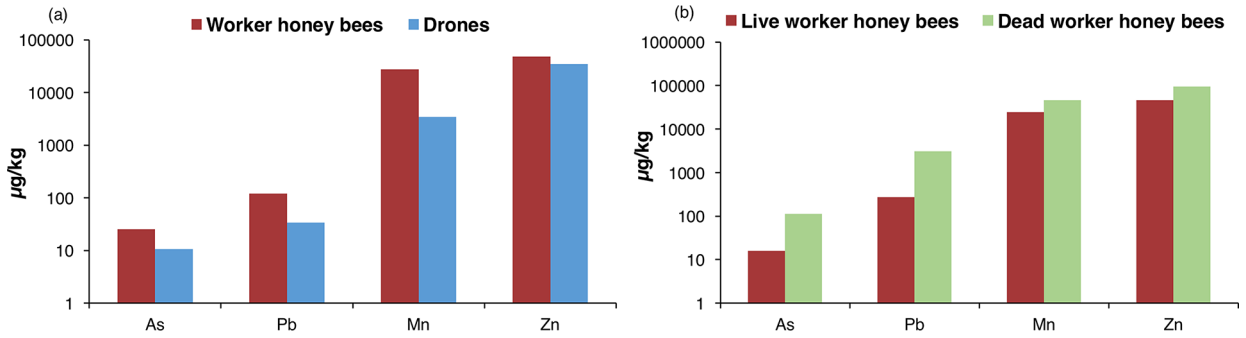

Figure 2. (a) Mean concentrations of trace elements As ((min−max) 24−26 µg/kg), Mn (15000−40000 µg/kg), Pb (79−160 µg/kg) and Zn (39000−58000) in worker honey bees (n = 60) and drones (As ((min−max) <10−21 µg/kg), Mn (2100−4800 µg/kg), Pb (26−41 µg/kg), and Zn (31000−38000)) (n = 60) collected from the coastal residential areas of Coogee (n = 30) and Randwick (n = 30) in November 2015. Trace element concentrations between the two honey bee types were not significantly different (p = 0.284−0.404, two sample t tests). (b) Mean concentrations in live worker honey bees (n = 60) of trace elements As ((min−max) 14−18 µg/kg), Mn (22000−27000 µg/kg), Pb (250−290 µg/kg), and Zn (39000−54000 µg/kg) concentrations and in dead worker honey bees (n = 60). As ((min−max) 66−160 µg/kg), Mn (19000−73000 µg/kg), Pb (3100−3100 µg/kg), and Zn (41000−150000) collected from the city area of Surry Hills in November 2015 (n = 30) and April 2016 (n = 30). Only the Pb concentrations were significantly different between live and dead worker bees (p < 0.005, two sample t test). Full data set is available in Table S1.

levels (p = 0.005−0.572, two-sample t tests) of all trace elemental concentrations than live worker honey bees, indicating contamination from the surrounding environment (Table S1 and Figure 2). However, only lead concentrations were significantly higher (p < 0.005, two-sample t test) in dead compared with live worker honey bees (3100 µg/kg vs 270 µg/kg) (Figure 2). A separate analysis of individual live worker bees from a single sample collected from Sydney CBD and Surry Hills sites showed that the mean Pb concentrations were 188 µg/kg with RSD 26% (range from 140−260 µg/kg) and 251 µg/kg with RSD 31% (range from 110−430 µg/kg) at each site, respectively.

Trace Element Concentrations in Beehive Products, Soil, and Dust. Mean concentration data for As, Mn, Pb, and Zn in honey bees (µg/kg), honey (µg/kg), wax (µg/kg), soil (mg/kg), and dust (mg/kg) are provided in full in Table S2 and are summarized below.

The highest mean concentrations of Pb in honey bees, honey, and wax were recorded in Broken Hill samples (2570, 295, and 11600 µg/kg, respectively). By comparison, Pb in honey from the nine Sydney sites did not exceed 22 µg/kg (Table S2). Concentrations of As in honey bees (160 µg/kg) and wax (77 µg/kg) were higher in Broken Hill compared with Sydney. Across Sydney, mean concentrations of Pb in honey bees were lowest (50 and 56 µg/kg) in the locations adjacent to national parks (Galston and Gordon) followed by the coastal residential areas of Coogee and Randwick (125 and 146 µg/kg). Honey bees sampled in city and industrial areas of Sydney contained higher Pb concentrations: Sydney CBD/Surry Hills/Newtown 230−440 µg/kg; inner west residential and industrial area of Marrickville 150−350 µg/kg and the mixed (industrial, commercial and residential) land use area of Mascot 418 µg/kg. Concentrations of Mn and Zn in beehive products (wax) were higher proximal to industrial locations, with the highest Mn and Zn values recorded in Broken Hill honey at 6280 µg/kg and 2180 µg/kg, some five and three times greater than at sites adjacent to national parks (Gordon: Mn 1320 µg/kg, Zn 800 µg/kg; Galston: Mn 4580 µg/kg, Zn 465 µg/kg).

Trace element concentrations in soil and dust were typically lower at background Sydney sites compared to Sydney metropolitan sites, with the highest values recorded at Broken Hill (Table S2). Elevated trace element concentrations in the Broken Hill beehive samples is consistent with high levels of contaminants found in the city's soils and dusts.[46,47] Soil Pb at the sample site in Broken Hill was 3290 mg/kg compared with 20−810 mg/kg at the Sydney sample sites (Table S2). Soil Pb from the city and inner west areas of Surry Hills, Newtown, and Marrickville exceeded the Australian Health Investigation Level (HIL-A) of 300 mg/kg applicable to residential dwellings,[56] corresponding with other studies of soil contamination in Sydney.[28,57−61] Maximum concentrations of As (23 mg/kg), Mn (1450 mg/kg), and Zn (3890 mg/kg) were found in Broken Hill soils but were below the Australian HIL guidelines for residential properties of 100, 3800, and 7400 mg/kg, respectively.[56]

Maximum dust Pb concentration was 4360 mg/kg in Broken Hill, 115 times greater than at the Galston site (38 mg/kg), which is adjacent to a national park. Similarly, the highest mean values of dust Mn and Zn were from Broken Hill: Mn 3130 mg/kg and Zn 7570 mg/kg. Sydney metropolitan soil trace element values were much lower at 104−700 mg/kg for Mn, and 307−590 mg/kg for Zn. Dust As concentrations were markedly greater at Surry Hills (230 mg/kg) and Broken Hill (124 mg/kg) than at other sampling sites across Sydney (2−77 mg/kg).

Pearson correlations were analyzed between trace element concentrations ($\log_{10}$ transformed) in beehive products and co-located samples of soil and dust (Table S3a−d). Of the trace elements analyzed, Pb had the most robust relationships among the trace elements and variables examined. Dust Pb was highly correlated to corresponding soil values (r = 0.806, p = 0.005, Figure 3a), with it being the most reliable predictor of outcomes variables. For example, only Pb concentrations in honey bees were significantly related to all corresponding concentrations in soil (r = 0.674, p = 0.033) and dust (r = 0.882, p = 0.001) (Figures 3b, c) as well as honey (r = 0.882, p = 0.003) and wax (r = 0.789, p = 0.007). Further, Pb in honey was significantly correlated (Pearson's r) to co-located soil (r = 0.716, p = 0.020) and dust Pb (r = 0.786, 0.007, Figure 3d,e). Wax Pb concentrations were correlated to dust Pb (r = 0.730, p < 0.017; Figure 3f) but not soil (Table S3a).

Zinc soil and dust values were correlated at lower levels compared to those in Pb (r = 0.634, p = 0.049), while soil and dust concentrations in As (r = 0.138, p = 0.704) and Mn (r = 0.547, p = 0.102) were not statistically significant. As per

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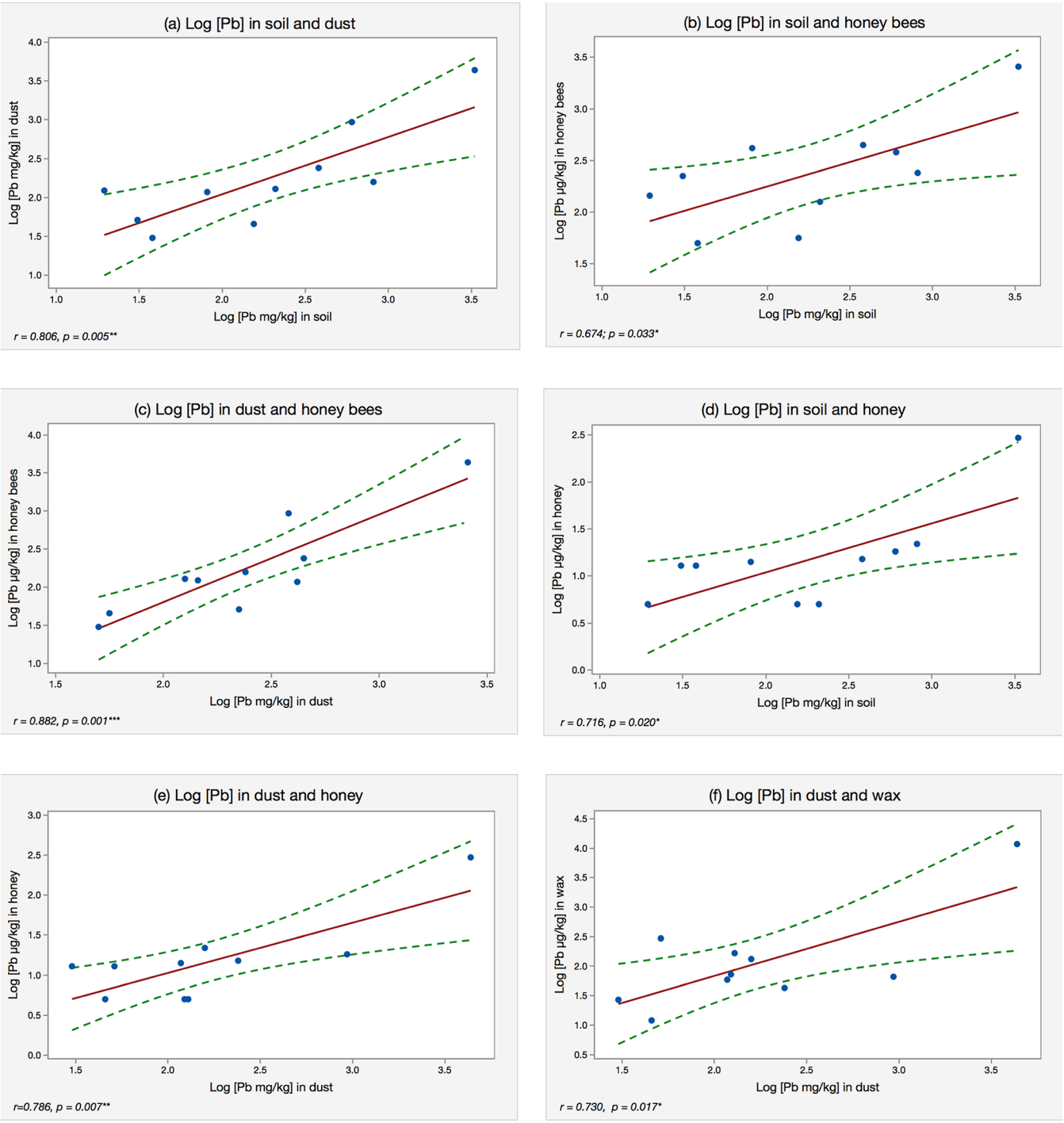

Figure 3. (a–f) Plots showing the relationship (with 95% confidence intervals) between $\log_{10}$ transformed mean Pb concentrations in honey bees (μg/kg, n = 39), honey (μg/kg, n = 17), wax (μg/kg, n = 16), and co-located soil (mg/kg, n = 44) and dust (mg/kg, n = 41), as well as the correlation between co-located soil Pb and dust Pb samples. Pearson correlation significant at the *0.05, **0.01, and ***0.001 level.

Pb, Zn in honey and wax were significantly correlated with co-located soil and dust concentrations (r = 0.725, p = 0.018; r = 0.725, p = 0.018), respectively. The statistical relationships for the remaining trace elements analyzed (As, Mn, and Zn) and their corresponding soil or dust values are provided in Tables S3a–d. Arsenic concentrations in honey and wax were below the NMI's LOR of 10 μg/kg, limiting statistical analysis of its relationship to the corresponding variables.

Lead Isotopic Compositions. Mean values of Pb isotopic compositions (^{206}Pb/^{207}Pb, ^{208}Pb/^{207}Pb) in honey bees, honey, wax, soil, and dust were compared to published values of the Broken Hill ore body, Sydney background values (subsurface soils and rocks representing geogenic values), and aerosols collected during the period when leaded petrol was consumed (Figure 4).[46,62−65]

The data show that Broken Hill samples are distinct from Sydney samples. Source apportionment modeling[66] showed that beehive samples from Broken Hill and corresponding environmental sample Pb isotopic compositions were within 95–98% of the local ore body (Figure 4; Table S5). The mean

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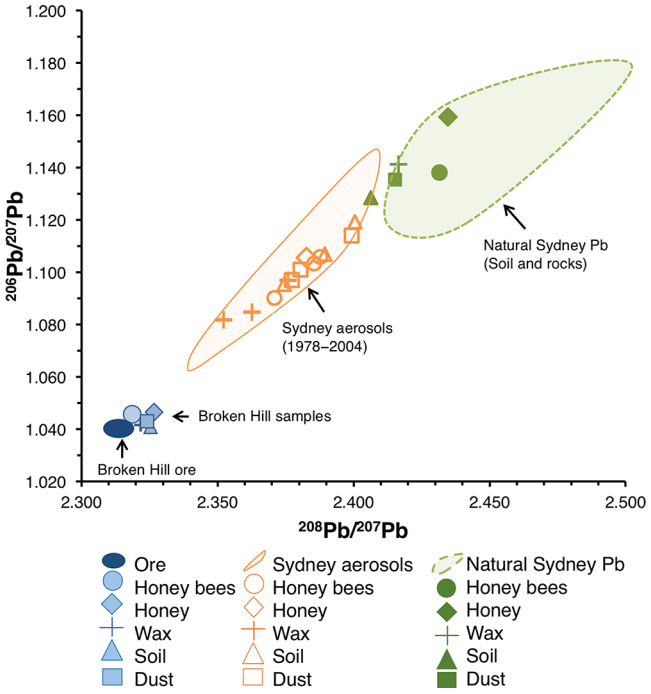

Figure 4. Mean Pb isotopic compositions for honey bees (n = 26), honey (n = 6), wax (n = 6), soil (n = 42), and dust (n = 35). Lead isotopic compositions for Sydney background soils, rocks, and aerosols were obtained from Wu et al.[62] Broken Hill ore body data were obtained from Kristensen and Taylor[46] and Gulson.[64]

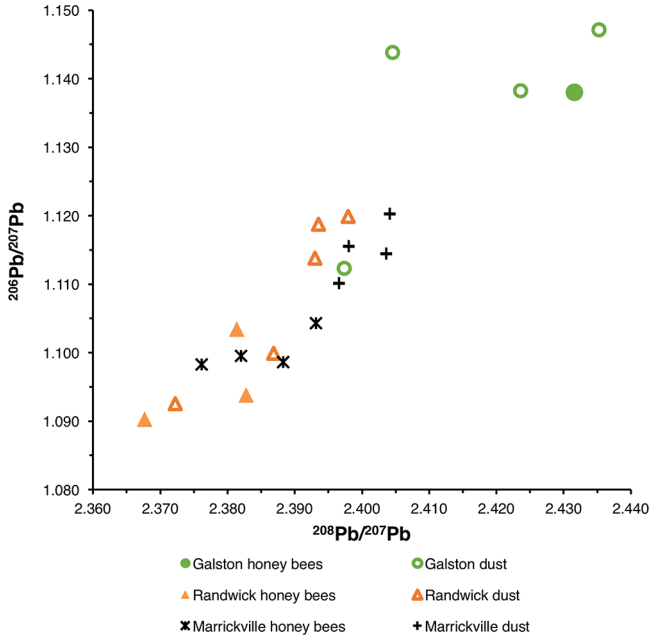

Figure 5. Lead isotopic compositions for individual site samples of honey bees (n = 8) and dust (n = 13) from Galston (Sydney background area), Randwick (coastal residential area), and Marrickville (residential–industrial area).

Pb isotopic compositions of honey bees, wax, and honey ($^{206}Pb/^{207}Pb$ 1.138–1.159; $^{208}Pb/^{207}Pb$ 2.417–2.435) and co-located dust from sites adjacent to Sydney national parks had Pb isotopic compositions ($^{206}Pb/^{207}Pb$ = 1.123–1.176, $^{208}Pb/^{207}Pb$ = 2.413–2.500) corresponding closely to those in background geogenic soil and rocks ($^{206}Pb/^{207}Pb$ 1.098–1.176; $^{208}Pb/^{207}Pb$ 2.388–2.500) (Table S5, Figure 4). Surface soils from the background sites were close to the "leaded petrol" aerosol envelope indicating adulteration of natural values by depositions from former petrol emissions, consistent with other Australian Pb isotopic composition analysis of soils distant from cities.[67,68] The mean Pb isotopic compositions of Sydney metropolitan samples (honey bees, honey, wax, soil, and dust) correspond to the Pb isotopic composition of Sydney aerosols (1978–2004) collected during a time period dominated by leaded petrol consumption, which ended in 2002.[17,62] However, the distribution of Pb isotopic composition values available for each site reveals there is some variation in Pb sources (Figure 5; Table S5). This is not surprising given the inherent variability of likely Pb sources in a large city such as Sydney (i.e., petrol, paint, industrial emissions and related sources), coupled to the fact that the honey bees forage over an area greater than 7 km^2.

DISCUSSION

Marginally higher concentrations of trace elements in worker honey bees compared to drones in this study are consistent with those in Mihaly Cozmuta et al.'s Romanian study[69] that found worker honey bees had approximately twice the Pb concentrations of drones. Exposure to environmental contaminants in worker honey bees is anticipated to occur during foraging activity outside of the hive, where atmospheric and substrate particles can attach to their body hair.[13] Drones usually have three or less flights per day, totaling about 30 min, around the beehive.[70] By contrast, a worker honey bee has about 10 flights per day, totaling up to 90 min and traveling up to 13 km, foraging for nectar and pollen.[71] Increased lifetime exposure to environmental contaminants in worker honey bees is corroborated by Figure 2, which shows statistically higher levels of Pb in dead worker honey bees than those alive (two-sample t test, p = 0.005) at the time of sampling (Table S1, Figure 2). Analysis of individual live honey bees from the CBD and Surry Hill in Sydney city showed that contamination is highly variable between individual insects. Given it is not possible to determine the age of the honey bees, we suggest that differing levels of Pb contamination may be a suitable proxy for their age. This inference is supported by the fact that worker honey bees are more contaminated than drones, which can only occur from their greater activity outside the hive where contamination is more prevalent. Thus, the data implies that worker honey bees are either accessing places consisting of variable Pb levels and/or are being contaminated as they age. This latter conclusion is not only consistent with other studies[72] but is further evidenced by the fact that dead bees at the Surry Hills site had statistically higher levels of Pb than live specimens from the same hive (Figure 2b). The issue of honey bee Pb contamination warrants further study to ascertain if it results in impairment of their function as they age.

Arsenic was below the LOR (<10 μg/kg) in all honey samples collected and analyzed in this study (Table S2). Although the highest As concentration in honey bees was found in Broken Hill (160 μg/kg) where elevated levels of atmospherically deposited dust trace elements are prevalent,[47,73,74] it was not detected in the corresponding honey sample (Table S2). This is consistent with the study by Alvarez-Ayuso and Abad-Valle[40] who showed there was limited transfer of As from honey bees to their honey. The physiological mechanism for the reduced transfer of As and other trace element contaminants in honey is not well-established but may be related to the honey bees'

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internal biological filtering[42,72,75−78] with some of the contaminants being excreted through its fecal mass[79] or its wax.[80]

Measurable concentrations of As in wax from Sydney CBD (14 μg/kg) and Broken Hill (77 μg/kg) are likely to reflect environmental contamination from dense traffic movements and mining activities, respectively.[72,81] Higher As concentrations in wax compared with honey[75,82] may be a result of the honey bees' process of producing and secreting wax[80] and its longevity in the hive versus that of honey. Typically, the wax comb is retained for reuse after seasonal honey production has been harvested.

Honey bees and their products have previously been shown to be linked to sources of environmental Pb, but the specifics of the spatial and temporal relationships remain poorly explained.[83−85] In our study, the following Pearson correlations were found between Pb in honey bees (r = 0.882, p = 0.001), honey (r = 0.786, p = 0.007), wax (r = 0.730, p = 0.017), and co-located dust concentrations (Figure 3c,e,f; Table S3a−d). Given that dust Pb is partly derived from surface soils (Figure 3a),[86] this indicates that honey bees and honey have potential application for monitoring the resuspension of soil Pb in the environment (Figure 3b,d).

The absence of a significant correlation between Mn and Zn in honey bees and co-located soil (r = 0.511, −0.587, p = 0.131, 0.074, respectively) and dust (r = 0.137, −0.731, p = 0.705, 0.016) (Table S3a−d) could be because Mn and Zn are essential elements for honey bee development and are more readily absorbed and secreted.[87,88] The honey bee's physiological system is likely to be better adapted to processing these elements compared to As and Pb, for which there are no biological uses due to their toxicity.[89,90] This interpretation is reinforced by the data in Table S2, which shows Mn and Zn concentrations are two times higher in dead honey bees versus live ones, whereas As and Pb concentrations are seven and 11 times, respectively, higher in dead specimens than those alive at the time of sampling.

The use of honey bees, honey, and wax as environmental proxies for discriminating sources at a larger scale appears to have some validity, particularly where land use types are markedly different. For example, the study data indicates that Pb, Mn, and Zn concentrations in honey bees and their products vary sufficiently to discriminate between uncontaminated areas adjacent to national parks (Galston, Gordon), Sydney metropolitan areas (Coogee, Randwick, Sydney CBD, Surry Hills, Newtown, Marrickville, Mascot), and the Pb−Zn−Ag mining city of Broken Hill. Even after excluding Broken Hill data, the Pearson correlation between Pb concentrations in Sydney metropolitan worker honey bees and corresponding dust samples remained significant (r = 0.731, p = 0.025). By contrast, analysis for associations of Pb concentrations between other paired data (i.e., those in beehive products and corresponding environmental samples) was not significant after the removal of the Broken Hill data (r = −0.022−0.650, p = 0.058−0.955). Although a relationship between Sydney metropolitan Pb concentrations in dust and soil was evident, it was marginally statistical insignificant (r = 0.650, p = 0.058). Previous studies of environmental contaminants within the Sydney metropolitan region reveals that multielemental concentrations in soil and dust across Sydney metropolitan suburbs are heterogeneous.[28,91−93] Given that honey bees forage an area over 7 km^2,[11,12,75] it likely that concentrations of trace elements found in honey bees and their products reflect a more generalized proxy of contamination at a suburb level. This would have the effect of smoothing variations in anthropogenically sourced contaminants measured at the different Sydney metropolitan suburb sites. Local geogenic factors that could influence the Pb isotopic composition of honey bees and their products is vastly different to low radiogenic Broken Hill type ores that were used predominantly in Australian leaded petrol and have contaminated Sydney soils and dusts.[52,68] The Pb isotopic composition of honey bees, honey and wax from Sydney metropolitan sites (^{206}Pb/^{207}Pb = 1.082−1.106, ^{207}Pb/^{208}Pb = 2.352−2.388), match closely the Pb isotopic compositions of aerosols (^{206}Pb/^{207}Pb = 1.067−1.148, ^{207}Pb/^{208}Pb = 2.341−2.410) collected from 1978−2004 (Table S5, Figure 4), the period when peak leaded petrol emissions were beginning to decline.[17] Several studies have shown that aerosols collected during the period of leaded petrol consumption correspond closely to the Pb used in petrol at that time,[68,94−96] and it is apparent that its signature remains.

In terms of geogenic sources, bedrock geology across the Sydney basin is composed predominantly of rocks and sediments of Triassic age or younger,[97] is more radiogenic than former lead petrol emissions (Figure 4), and corresponds more closely to samples from background areas. Since the withdrawal of Australian leaded petrol in 2002, there remain only limited atmospheric lead emissions in the city of Sydney. The largest emitter in the city of Sydney was from a used lead acid battery (ULAB) approximately 2 km from the Mascot bee hive site, which reported 400 kg of Pb emissions to the air in 2015−2016.[98] Moreover, the ULAB site is also approximately 2 km from the Australian Nuclear Science and Technology Organisation's air quality station at Mascot and its emissions do not appear to influence seasonal or long-term downward trend of lead in air concentrations since before 2002 (Figure S1).[99−101] Annual Pb in air peaks occur during the cooler Australian months of June to September, with mean concentrations from 2015 at 6.14 ng/m^3 and monthly maximum average concentrations not exceeding 15 ng/m^3; Figure S1). In the warmer months of the sample period (November 2015 to April 2016) when honey bees are foraging intensively, Pb in air was <5 ng/m^3; Figure S1). Despite the reducing contemporary atmospheric Pb sources in Sydney, the study indicates that honey bees, honey and wax have good potential application for their use as environmental indicators. This is particularly the case where there are markedly different sources such as natural background, urban-residential (legacy) and mining, and where Pb is the focus trace element.[31] However, further work applying the same approach to other areas is required to confirm the wider applicability of this approach.

The data show that honey bees and their products are suitable markers of contemporary contamination emanating from mining operations in Broken Hill where total Pb emissions to the atmosphere over 2015−2016 were estimated at 28000 kg.[98] Lead in air concentrations (as total suspended particulates) some 1.6 km from the Broken Hill honey bee sampling site averaged 185 ng/m^3, with a maximum of 527 ng/m^3 between January 2015 and April 2016 (Figure S1).[102] In contrast to Sydney, peak Pb in air concentrations in Broken Hill occur in the Australian summer months when honey bees are most active (Figure S1). Given the significant current Pb and other dust contaminant emissions in Broken Hill,[47,73,74] it is not surprising that trace element concentrations from Broken Hill samples were tens to hundreds of times greater than those from Sydney background sites adjacent to national parks (Table S2). The sensitivity of honey bees and their products as environmental markers is supported by the fact that the Pb

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isotopic compositions of samples from Broken Hill were within 95−98% of the Broken Hill ore body (Figure 4). Other studies of Broken Hill contamination have shown that ambient dust is similar in its Pb isotopic composition to the Broken Hill ore body and that Pb isotopic composition analysis of ambient dust is an effective analytical tool for discriminating sources of contamination in that city.[47,73,103,104]

Recent studies of Australian soils,[45] sediments,[105,106] wines,[68] peat bogs,[107] seagrass,[108] lichens,[62,109] and wildfire ash[110] have all been shown to contain varying proportions of legacy petrol Pb with a Broken Hill signature, indicating its widespread significance as a contaminant. Moreover, Broken Hill Pb has been found across the globe due its former common use in leaded petrol.[50,51,111,112] Although Pb emissions from petrol have also been eliminated in Australia and its adverse health effects diminished, it remains a persistent contaminant in the environment from its seven-decade period of use between 1932 and 2002.[17,52,113] Therefore, in the absence of other contemporary Pb sources in Sydney, the data in this study indicate that legacy Pb isotopic compositions in contemporary honey bees and their products reflect the dominant source of Pb in the ambient environment. Recent analysis of Sydney aerosols before, during, and after wildfires show that Pb compositions have shifted back toward natural values following the cessation of leaded petrol use in 2002.[52] However, assessment of ash produced during wildfires[110,114] contemporary lichens,[62] wine,[68] and now in honey bees and their products demonstrate how easily legacy Pb is being recycled into food and ecological systems. Such persistence should act as a warning to the use and unregulated release of other chemicals that may be harmful to humans and ecosystems.[115]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04084.

Supplementary tables and figures detailing trace element and statistical analyses, Pb isotopic compositions in honeybees, honey, wax, soil and dust, and current Pb sources in Sydney and Broken Hill, Australia (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mark.taylor@mq.edu.au.

ORCID

Xiaoteng Zhou: 0000-0002-1900-6037

Mark Patrick Taylor: 0000-0001-7598-9982

Author Contributions

X.Z. helped develop the project design and undertook the majority of field sampling, laboratory analysis, data assessment, and write up. M.P.T. devised the original project idea and design, assisted with field sampling, and undertook data analysis, interpretation, and write up. P.D. and S.P. assisted with sample design and data write up.

Notes

The authors declare no competing financial interest.

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