

# Soil lead contamination decreases bee visit duration at sunflowers

Frances S. Sivakoff<sup>1</sup> · Mary M. Gardiner<sup>1</sup>

Published online: 20 April 2017  
© Springer Science+Business Media New York 2017

**Abstract** Legacies of lead contamination present challenges in the management of urban greenspaces for beneficial insect conservation. In particular, the sublethal effects of lead contamination on bee foraging behavior could negatively impact plant-pollinator interactions and the sustainability of urban agriculture. It is difficult, however, to distinguish between differences in foraging behavior caused by lead contamination directly as compared to differences resulting from variation in floral traits, which can also be affected by contamination. We compared the foraging behavior of bees, specifically the number of visits and visit duration, at sunflowers grown in lead-contaminated and uncontaminated soils. We also measured soil lead contamination's effects on sunflower morphological traits. While the number of visits a sunflower head received was not affected by soil lead contamination, bee visit duration was shorter at sunflowers grown in lead contaminated soil. This effect of lead contamination on visit duration was not mediated by sunflower floral traits, which were themselves affected by lead contamination. The inability of bees to distinguish between sunflowers grown in contaminated vs. uncontaminated soil prior to visitation suggests a possible bioaccumulation pathway for lead in bees.

**Keywords** Foraging behavior · Lead contamination · Pollination · Structural equation modeling · Urban agriculture

**Electronic supplementary material** The online version of this article (doi:10.1007/s11252-017-0674-1) contains supplementary material, which is available to authorized users.

✉ Frances S. Sivakoff  
sivakoff.3@osu.edu

<sup>1</sup> Department of Entomology, The Ohio State University, Columbus, OH 43210, USA

## Introduction

Urban greenspaces, including public and private lands, right-of-ways, and vacant land, can be managed to conserve beneficial species and the ecosystem services they provide (Gardiner et al. 2013, 2014). While the transformation of urban greenspaces holds potential, the landscape legacies of these habitats represent a potential threat to the biota we seek to conserve. Cities across the United States, and in particular those with industrial pasts, often have elevated soil lead (Pb) levels resulting from the historic use of Pb-based paint and leaded gasoline, the close proximity of industrial smelters and coal-fired plants, and the improper management of industrial waste (Mogren and Trumble 2010; Singh and Prasad 2011; Kohrman and Chamberlain 2014). Lead is a threat to human health (reviewed by Jarup 2003), and a possible exposure pathway is through growing food in contaminated soils (Brown et al. 2016). The rapid growth and popularity of urban agriculture (Mok et al. 2014) has increased awareness of the problem of Pb contamination in urban soils. To reduce the risk of exposure to Pb, urban growers generally confirm that their arable soils have Pb levels below a threshold level, which ranges from 50 ppm – 500 ppm in the United States depending on location and regulatory agency (Jennings and Petersen 2006). Importantly, determining that a site's soil is below a regulatory threshold does not indicate the site is Pb-free or that the Pb present does not affect the structure and function of the plant and animal communities at that site.

Bees are important bioindicators of a variety of environmental contaminants, including radiation, chemicals used as pesticides, and heavy metals (Bromenshenk et al. 1985; Kevan 1999; Devillers and Pham-Delègue 2002; Parmentier et al. 2014; Pisa et al. 2015; Bargańska et al. 2016). Individuals can be exposed to contaminants through multiple routes; bees can acquire contaminants through the

consumption of contaminated nectar and/or collect contaminated dusts on their bodies from either direct sprays (in the case of pesticides) or by contacting contaminated surfaces (Pisa et al. 2015). The majority of studies focusing on pollinators and heavy metals have used the European honey bee (*Apis mellifera*) or its honey as bioindicators (e.g. Free et al. 1983; Leita et al. 1996; Perugini et al. 2011; van der Steen et al. 2012; Satta et al. 2012; Al Nagggar et al. 2013; Ruschioni et al. 2013). Other bee species are likely affected by heavy metals in other ways given differences in morphology, nest location, and colony structure. Given the importance of wild pollinators in urban agroecosystems (Matteson et al. 2008; Stavert et al. 2016), an understanding of Pb contamination's effect on both wild and managed pollinators is crucial for the sustainability of pollination services.

The accumulation of heavy metals in plant parts like pollen and nectar exposes visiting pollinators to contamination and alters pollinators' foraging behavior (Meindl and Ashman 2014), but this sublethal effect is poorly understood. This is in part because pollinators' behavioral responses varies by contaminant. For example, pollinators visited nickel-contaminated floral resources less frequently and for shorter periods of time (Meindl and Ashman 2013, 2014). Dissimilarly, neither honey bee foraging behavior nor subsequent pollen deposition was affected by high floral selenium concentrations (Hladun et al. 2013). Additionally, it is difficult to identify the sublethal effects of heavy metals on plant-pollinator interactions because we must disentangle the direct effect of heavy metal contamination on pollinator behavior with the indirect effect of that contamination as mediated by plant physical traits. High concentrations of heavy metals in the soil can reduce floral size (Antonovics et al. 1971; Hladun et al. 2011). Because bees prefer large floral displays and have increased likelihood of within-plant movement at these displays (Mitchell et al. 2004), it is difficult to determine whether differences in pollinator foraging are the result of heavy metal contamination or floral traits.

We assessed the effects of soil Pb contamination on foraging behavior, specifically the number of visits that a bee makes to a particular flower and the duration of those visits, at potted sunflowers (*Helianthus annuus* L.) grown in either Pb-contaminated or uncontaminated potting soil. Sunflowers are common in urban agroecosystems and are important nectar sources for pollinators and food sources for birds and they are also hyperaccumulators of Pb (Adesodun et al. 2010; Cutright et al. 2010). While the majority of Pb is taken up by sunflowers' leaves and stems (Boonyapookana et al. 2005), Pb has also been detected in sunflower honey collected from apiaries in polluted areas (Citak et al. 2012). We first measured soil Pb contamination's effect on sunflower physical traits, which we predicted based on the literature (Antonovics et al. 1971) would be altered by Pb contamination. We then evaluated the frequency and duration of bees'

visits to sunflowers and predicted that sunflowers grown in Pb-contaminated soil would receive fewer bee visits and the duration of those visits would be shorter compared with sunflowers in uncontaminated soil. We used structural equation modeling (SEM) to evaluate the relative importance of the direct effects of soil Pb contamination compared to the indirect effects of soil Pb contamination as mediated by sunflower physical traits on flower visit duration.

## Methods

### Soil amendment and plant growth

To create Pb-contaminated soil, we added lead nitrate,  $\text{Pb}(\text{NO}_3)_2$ , to thoroughly wetted, peat-based growing medium (Pro-Mix HP, Premier Tech Horticulture) at a concentration of  $80 \text{ mg Pb kg}^{-1}$  (weighed prior to wetting the medium). This contamination level is the maximum allowable concentration of Pb permissible at urban farming sites in California (California Office of Environmental Health 2010). We chose this threshold because it is among the most conservative in the country, and we were interested to see if we would observe sublethal effects of Pb contamination even at a low concentration. We added our thoroughly mixed, amended soil to 6 in. plastic pots ( $n = 50$ ) and watered these pots at 45% of the soil water-holding capacity for at least 9 days (9–14 days) to allow the amended soil to equilibrate before we transplanted seedlings into the pots. We also created control pots ( $n = 34$ ), which consisting of uncontaminated wetted, peat-based growing medium that was potted and watered in the same manner as Pb-amended soil.

We germinated sunflowers variety 'Dwarf Sunspot' individually in thoroughly wetted, peat-based growing medium and transplanted seedlings once they produced their first pair of true leaves into pots containing either contaminated or control soil. Transplanted seedlings (14–19 day post germination) in their respective soil contamination treatments were randomly assigned a location in the greenhouse maintained at 22/20 °C, 16/8 h day/night. Plants were watered daily to maintain approximately 35% water holding capacity and were fertilized once during this growing period at a rate of 150 ppm (Peters Professional 20–10–20 General Purpose, Everris). Dwarf Sunspot sunflowers generally produce a single flower head, and once this flower head formed but before it opened, we covered the flower head with a tightly woven mesh bag (1 gal paint strainer) to exclude access to the flower head by pollinators and other insects in the greenhouse. We measured plant height at 49 days after planting (5 days before the start of the field experiment). Soil from plants not used in the field experiments ( $n = 8$  per soil contamination treatment) was air dried and analyzed for Pb by the Service Testing and Research Lab at the Ohio Agricultural Research and Development Center.

## Field experiment

To test the effect of soil Pb contamination on pollinator behavior, we placed five pairs of Pb-contaminated and control mature sunflowers in the field for approximately 6 h and recorded pollinator behavior. The pairs were arranged so that there was 1 m between the sunflowers in a pair and 10 m between pairs. We repeated this experiment on three total days with new plants used each day, for a total of 15 pairs of contaminated and control plants. Our experiments were conducted on mown turf at the Ohio Agricultural Research and Development Center campus (40°46′52.9″N, 81°55′51.7″W). At the start of the experiment, we carried bagged sunflowers from the greenhouse and removed the bags once all of the sunflowers were in place, at approximately 08:30 h. Once we removed the bags, pollinators were able to access sunflowers and we recorded each flower head for the entire period that sunflowers were accessible to pollinators (the “experimental period”; approximately 08:30–14:15) using a security camera system (Q-see, model no. QSC26404, Anaheim, CA) that stored videos for subsequent processing. Following the conclusion of the experimental period each day, we measured the width and took pictures of each flower head along with a scale bar to standardize the measurement scale in the images. We later processed these images to quantify the area of open flowers in each flower head using the image processing software *Fiji* (Schindelin et al. 2012).

Our study took place over the course of three days within a 276 m<sup>2</sup> area, and at the time of our study, there was an apiary consisting of six colonies of honey bees approximately 80 m north of our study site. It is likely that bumble bee and honey bee visitors to our site were not each arriving from an independent colony but instead that we were sampling the response of a given bee community nesting within the surrounding landscape. Different colonies could respond differently to experimental treatments based on factors such as previous exposure to heavy metals, and further work is needed to determine if this is the case.

## Video analysis

We reviewed each video to quantify the number of pollinator visits to that flower and the duration of each visit. We classified the start of a visit as the time that a pollinator entered the field of view and landed on the flower head and the end of the visit as the time that the individual left the flower head and the field of view. If an individual left the field of view and then immediately (< 2 s) returned, we classified this event as the same visit. Otherwise, each new appearance in the field of view was classified as a new visit. All pollinators were identified to the lowest taxonomic level possible for the video resolution (16 frames per second with a playback pixel

resolution of 352 × 240 and an aspect ratio of approximately 1.222:1).

## Statistical analysis

We assessed the effect of soil Pb contamination on both sunflower physical traits and the number and duration of bee visits in the statistical platform R 3.2.5 (R Development Core Team 2016). We first evaluated the effect of soil contamination on sunflower physical traits. Differences in plant height, flower head area, and the proportion of open flowers in the flower head between sunflowers grown in contaminated vs. uncontaminated soil were assessed using Welch Two Sample t-tests. Flower head area was log transformed and proportion of open flowers was arcsin transformed prior to analysis to meet assumptions of normality.

We assessed the effect of soil Pb contamination on foraging behavior in the three most commonly observed groups from our video recording (Table 1): bumblebees (*Bombus* spp.), honey bees, and bees from the family Megachilidae. Separately for each taxa, we evaluated whether soil Pb contamination affected the number of bee visits using negative binomial generalized linear models with the *glm.nb* function in the MASS package (Venables and Ripley 2002). In these models, soil contamination, flower head area, date, and the interaction between soil contamination and flower head area were included as fixed effects. As the recording time at each flower varied from problems with the recording hardware (median = 20,129 s, min = 11,806, max = 21,175), the total recording time at a particular flower was included in these models as an offset. We used likelihood ratio tests to determine if the interaction between soil contamination and flower head area was significant and Wald type II chi-square tests using the *anova* function in the car package (Fox and Weisberg 2011) to evaluate the influence of the fixed effects.

To evaluate the effect of soil Pb contamination on visit duration we built a structural equation model using the lavaan package (Rosseel 2012). Given a hypothesized causal relationship among variables, SEM uses maximum likelihood to solve a set of structural equations and, for each pair of variables, deconstructs their correlation into direct and indirect effects (“causal components”) and unanalyzed contributions (“noncausal components”; Schemske and Horvitz 1988). Additionally, SEM can evaluate relationships between measured and latent variables, which are unobserved constructs defined by one or more measured variables. In our model, we included all of our measured sunflower physical traits (sunflower height, flower head area, and area of open flowers) to inform the latent variable of ‘floral traits’. This ability to use latent variables is beneficial for our study because a variety of sunflower-related factors (not all of which are measured) likely influence a bee’s foraging behavior. Our model hypothesized that bee visit duration was affected by soil Pb

**Table 1** Ten most commonly observed visitors to sunflowers by soil contamination

Uncontaminated Sunflowers				Pb-Contaminated Sunflowers		
Rank	Taxa		Count	Taxa		Count
1	Bumblebee	<i>Bombus</i> spp.	129	Megachilids	Family Megachilidae	127
2	Honey bee	<i>Apis mellifera</i> L.	112	Honey bee	<i>Apis mellifera</i> L.	107
3	Megachilids	Family Megachilidae	108	Bumblebee	<i>Bombus</i> spp.	84
4	Carpenter bee	<i>Xylocopa</i> spp.	26	Carpenter bee	<i>Xylocopa</i> spp.	32
5	Soldier beetle	Family Cantharidae	14	Sweat bee	Family Halictidae	18
6	Sweat bee	Family Halictidae	10	Yellow jacket	<i>Vespula</i> spp.	11
7	Hoverfly	Family Syrphidae	8	Longhorn bee	<i>Melissodes</i> spp.	10
8	Squash bee	<i>Peponapis</i> spp. or <i>Xenoglossa</i> spp.	8	Hoverfly	Family Syrphidae	9
9	Moth	Lepidoptera	4	Spotted cucumber beetle	<i>Diabrotica undecimpunctata</i>	7
10	Yellow jacket	<i>Vespula</i> spp.	4	Soldier beetle	Family Cantharidae	7

contamination and by floral traits. Because Pb soil contamination can have strong effects on plant morphology (Antonovics et al. 1971; Kastori et al. 1998), our model hypothesizes that the construct of floral traits was affected by soil Pb contamination (Fig. 1). Using SEM, we are able to assess both the direct effect of soil Pb contamination and its indirect effect as mediated by sunflower floral traits on bee visit duration. Prior to analysis, visit duration was log transformed to meet assumptions of normality. Separately for each species, we assessed the goodness of fit of the overall model and estimated the accuracy and significance of each path coefficient using the adjusted bootstrap percentile method. Coefficient estimates were based on 10,000 bootstrap replicates, and we calculated the 95% confidence intervals for each standardized estimate. We used the *parameter estimates* function in the lavaan package to calculate the indirect and direct effects, their bootstrapped estimates, and 95% confidence intervals.

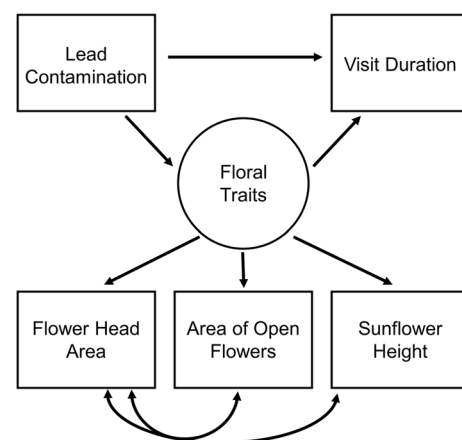
## Results

Chemical analysis of the soils confirmed that Pb-contaminated soil had higher concentrations of Pb compared to uncontaminated soil (average concentration  $\pm$  1 standard deviation =  $47.26 \mu\text{g/g} \pm 14.70$  for Pb-contaminated vs.  $3.53 \mu\text{g/g} \pm 0.63$  for uncontaminated). Sunflowers grown in Pb-contaminated soil were shorter ( $t = -5.34$ ,  $df = 27$ ,  $P < 0.001$ ; Fig. 2a) and had smaller flower heads ( $t = -7.81$ ,  $df = 22$ ,  $P < 0.001$ ; Fig. 2b) when compared to plants grown in uncontaminated soil. The proportion of open flowers on the day of the experiment, however, did not differ regardless of soil contamination ( $t = 0.10$ ,  $df = 27$ ,  $P = 0.92$ ; Fig. 2c).

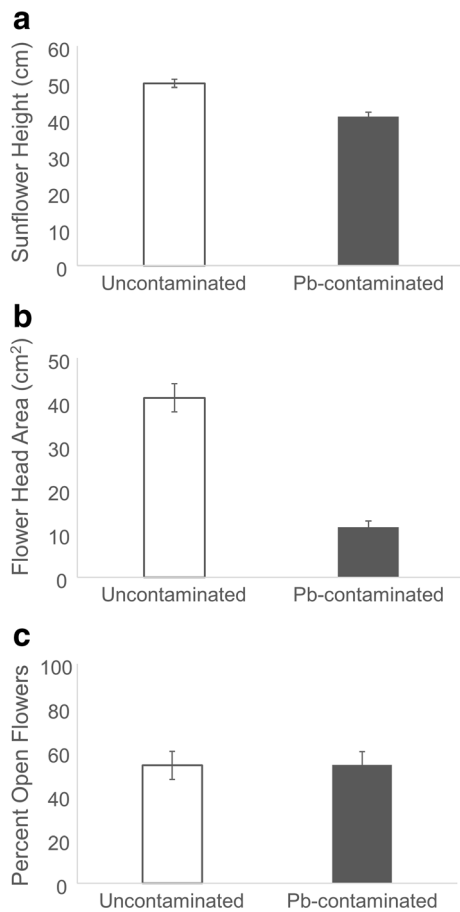
Soil contamination did not affect the number of visits by bumblebees ( $X^2 = 0.28$ ,  $df = 1$ ,  $P = 0.60$ ), honey bees ( $X^2 = 0.17$ ,  $df = 1$ ,  $P = 0.68$ ), or megachilids ( $X^2 = 0.51$ ,  $df = 1$ ,  $P = 0.47$ ). The effect of flower head area on the number of

visits was not significant (bumblebees:  $X^2 = 1.79$ ,  $df = 1$ ,  $P = 0.18$ ; honey bees:  $X^2 = 0.10$ ,  $df = 1$ ,  $P = 0.75$ ; megachilids:  $X^2 = 0.48$ ,  $df = 1$ ,  $P = 0.49$ ). The number of visits was different by day for both honey bees ( $X^2 = 8.09$ ,  $df = 2$ ,  $P = 0.02$ ) and megachilids ( $X^2 = 33.67$ ,  $df = 2$ ,  $P < 0.001$ ) but not for bumblebees ( $X^2 = 5.04$ ,  $df = 2$ ,  $P = 0.08$ ).

For bumblebees ( $X^2 = 1.08$ ,  $df = 2$ ,  $P = 0.58$ ) and honey bees ( $X^2 = 3.90$ ,  $df = 2$ ,  $P = 0.14$ ), our *a priori* model of Pb contamination's effects on floral traits and log-transformed visit duration converged and appeared to be a good fit for our data, with Comparative Fit Index (CFI) scores  $> 0.95$ , and standardized root mean squared residual (SRMR) scores  $< 0.08$  (Fig. 3 and Online Resource 1; Hu and Bentler 1999). For both groups, soil Pb contamination had a significant negative path coefficient to floral traits (bumblebees:  $-0.87$ , honey bees:  $-0.90$ ), indicating that soil Pb had a direct negative effect on sunflower morphology. These standardized path coefficients are measures of the predicted change (in standard deviations)

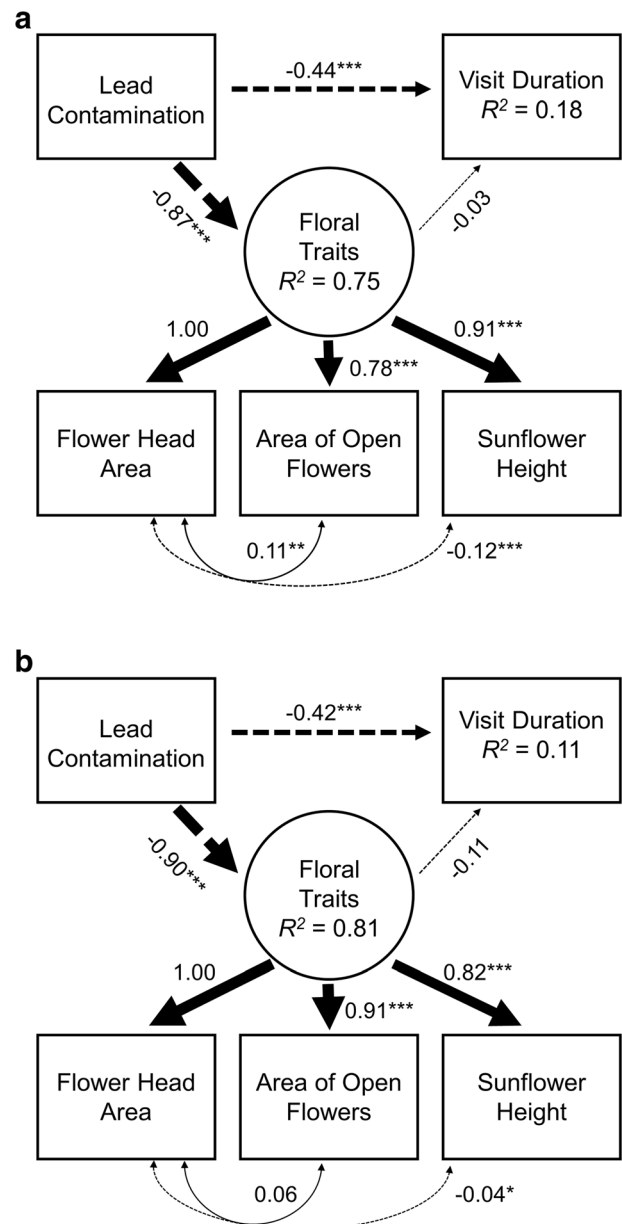


**Fig. 1** Hypothesized relationship between soil Pb contamination and bee visit duration. This model includes both the direct effect of soil Pb contamination and the indirect effect as mediated by floral traits. Variables enclosed by squares represent measured variables, while ‘floral traits’ (encircled) represents a latent variable



**Fig. 2** Plant morphological traits (mean ± SE) for plants grown in Pb-contaminated and uncontaminated soil: **a** sunflower height, **b** flower head area, and **c** proportion of open flowers in the flower head

in a response variable when we allow a single predictor variable to vary and hold all other correlated variables constant. For example, in the bumblebee model a  $-0.87$  standard path coefficient from Pb contamination to floral traits means that varying Pb contamination by one standard deviation resulted in floral traits decreasing  $0.87$  standard deviations. Soil Pb contamination, which in our models was the only variable affected floral traits, was highly predictive of sunflower floral traits, explaining  $75\%$  (bumblebee model) and  $81\%$  (honey bee model) of the variation. Soil Pb contamination also had a significant direct negative effect on log-transformed bee visit duration, with significant negative path coefficients for both the bumblebees ( $-0.44$ ) and honey bee ( $-0.42$ ) models (Table 2 and Fig. 3). This suggests that Pb contamination significantly reduces bumblebee and honey bee visit duration when floral traits are held constant. Neither the direct effect of floral traits (Fig. 3) nor the indirect effect of soil Pb contamination (acting through floral traits; Table 2) were significant. Altogether, little of the observed variation in log-transformed visit duration was explained by the direct and indirect effects of Pb contamination (bumblebee model:  $R^2 = 0.18$ , honey bee model:  $R^2 = 0.11$ ). This low variance explanation suggests



**Fig. 3** Solved path models for (a) bumblebees and (b) honey bees. Each line's thickness indicates the strength of the relationship, and the number associated with each line (standardized to range from 0 to 1) indicates the relative influence of that particular variable on the response variable when all other variables are held constant. Visit duration was log-transformed prior to analysis. Positive effects are represented by solid lines, negative effects by dashed lines. For single-headed arrows, these values are standardized path coefficients, for double-headed arrows, correlation estimates between predictor variables. Indirect effects are calculated by multiplying the standardized path coefficients of the constituent direct effects together. When this relationship is significantly different from zero, statistical significance is designated with an asterisk (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P \leq 0.001$ )

that additional unmeasured factors and/or random variation influenced bee visit duration.

The fit of our *a priori* model to the Megachilidae data was not as definitive as it was for the other two bee taxa (Online

**Table 2** Direct and indirect (acting through floral traits) effects of soil Pb contamination on bee visit duration for each of the three bee taxa as quantified by SEM. Path diagrams for bumblebees and honey bees are illustrated in Fig. 3 and path diagram for Megachilidae not shown (see *Results* for details)

Group	Parameter	Standardized Estimate	z-statistic	p-value	Standardized Lower 95% Confidence Interval	Standardized Upper 95% Confidence Interval
Bumblebee	Direct	-0.44	-2.92	0.004	-0.66	-0.22
	Indirect	-0.02	-0.37	0.708	-0.13	0.19
Honey bee	Direct	-0.42	-3.11	0.002	-0.69	-0.16
	Indirect	0.10	0.82	0.412	-0.17	0.37
Megachilidae	Direct	-0.29	-1.43	0.152	-0.59	0.16
	Indirect	-0.09	-0.44	0.662	-0.53	0.21

Standardized confidence intervals calculated by bootstrap ( $N = 10,000$ ). Intervals that overlap zero are considered non-significant

Resource 1). While the two-index presentation strategy (Hu and Bentler 1999) would suggest that our model fit well, with both a CFI score of 0.99 and a SRMR score of 0.05, the  $X^2$  goodness-of-fit statistic was highly significant ( $X^2 = 20.75$ ,  $df = 2$ ,  $P < 0.001$ ) and the Root Mean Square Error of Approximation (RMSEA) was  $>0.06$  (although this fit index has an inflated Type II error rate at sample sizes  $<250$ ). This questionable fit prompted us to examine the resulting path model for general trends only. In looking at the path coefficients, neither the direct effect of soil Pb contamination nor the indirect effect as mediated by floral traits had a significant effect on log-transformed visit duration (Table 2), although these path coefficients' signs and relative effect sizes were similar to the bumblebee and honey bee models.

## Discussion

Soil Pb contamination altered bee foraging behavior, and this effect was independent of plant height, flower head area, or the area of open flowers despite contamination's strong effect on these physical traits. Bees spent 5.4 (bumblebee), 3.7 (honey bee), and 3.6 (Megachilidae) times longer foraging at sunflowers grown in uncontaminated relative to Pb-contaminated soil. At the same time, the number of unique visits to a sunflower did not depend on soil contamination. This is similar to the effect of other heavy metals on plant-pollinator interactions (Se: Hladun et al. 2013; Ni: Meindl and Ashman 2014) and supports the assertion that foragers are unable to visually distinguish between resources grown in contaminated and uncontaminated soil (Meindl and Ashman 2013, 2014).

Soil Pb contamination clearly altered plant growth: contaminated soil sunflowers were 18% shorter and had flower head areas that were 38% smaller than sunflowers grown in uncontaminated soil. While we hypothesized that differences in plant morphology would affect bee foraging behavior, our finding that these floral traits did not mediate the effect of soil contamination on bee visit duration was contrary of our expectation. Our results instead suggest that bees are detecting

some variation in floral resources grown in contaminated soil after arrival and adjusting their visit durations based on their assessment in reward quality. While we did not measure Pb accumulation in nectar or pollen, and in general, exposure to Pb-contaminated soil is thought to be the most common exposure pathway (Brown et al. 2016), heavy metals can accumulate in floral rewards (Hladun et al. 2011; Meindl and Ashman 2014), Pb has been detected in honey (Citak et al. 2012), and the consumption Pb-contaminated nectar can have deleterious effects on bees (Di et al. 2016). Bioaccumulation through consumption has been documented in terrestrial food webs (reviewed by Gall et al. 2015), and if Pb is present in floral resources, then this inability to discriminate contaminated flowers prior to contact could lead to bioaccumulation within exposed populations. Alternatively, Pb could have affected visit duration through other aspects of nectar quality, including sugar content and nectar quantity, which could also negatively impact pollinator survivorship and reproductive success. Disentangling the mechanism(s) driving the behavioral change in pollinators when contacting resources grown in contaminated soil is key to advancing pollinator conservation and pollination services in urban greenspaces where elevated soil heavy metal concentrations are commonly found.

Importantly, the pollinators exposed to our experimental sunflowers were likely naïve to Pb-contaminated plants, as the site where we conducted the experiment is located in a rural landscape dominated by agriculture and forested habitats. We might expect that pollinators in urban ecosystems, with previous exposure to heavy metal contaminants, to behave differently at contaminated flowers. For example, pollinator communities at serpentine sites, characterized by soils with relatively high concentrations of several metals, visited potted *Streptanthus polygaloides* equally regardless of whether they were grown in Ni-treated or control soil. When evaluated at non-serpentine sites that hosts a closely-related, non-accumulator species, however, *S. polygaloides* grown in Ni-treated soil received 60% fewer bee visitors relative to controls (Meindl and Ashman 2015). This result suggests that the pollinator communities found at each of these sites differ in their

tolerance of heavy metal accumulation in floral rewards. If pollinator communities in urban ecosystem are in fact more tolerant of heavy metal contamination, then we would not expect differences in visitation rate between contaminated and uncontaminated plants. If this tolerance stems from the deterrence of less-tolerant pollinator species from contaminated areas, then we would expect pollinator richness and species composition might be negatively affected (similar to the “Elemental Filter Hypothesis”; Meindl and Ashman 2015). Alternatively, this tolerance could arise on the individual level after repeated exposure to contaminants. Further work is needed to evaluate which, if either, selective force is at play in urban ecosystems.

Our finding that soil Pb contamination affects pollinator foraging behavior can begin to inform the assessment of urban greenspaces for species conservation. In particular, if a site has high levels of Pb, then plantings established for pollinator conservation could take up this contaminant. Reduced foraging duration at these contaminated resources could result in reduced pollination success for plants and nutrition for pollinators that are potentially intolerant of Pb-contaminated floral resources. Additionally, if pollinators are unable to detect contaminated floral resources prior to contact, as we found here, then Pb could bioaccumulate in pollinators as a result of contact with or consumption of contaminated pollen or nectar. This has implications not only for the use of hyperaccumulators as nectar sources to sustain pollinators in urban agriculture, but also, as suggested by Meindl and Ashman (2013, 2014), for the use of insect-pollinated plants in phytoremediation. Further work to establish the bioaccumulation pathway of Pb and other heavy metals for pollinators and determine the effects of chronic exposure on pollinator health and efficiency will be necessary to evaluate the full impact of heavy metal contamination on pollination services and whether it is necessary to consider this contamination in urban greenspace design and management.

**Acknowledgements** We thank Nicole Hoekstra and Chelsea Gordon for their assistance in the field and Rachel McLaughlin for reviewing videos. Charles Goebel and Robert Gates provided guidance in statistical analyses and Riccardo Bommarco provided helpful comments on the manuscript. Funding support provided by the National Science Foundation Early Career Development Program (CAREER-1253197) to M.M.G.

## References

- Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA (2010) Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. *Water Air Soil Pollut* 207:195–201
- Al Naggar YA, Naiem E-SA, Seif AI, Mona MH (2013) Honey bees and their products as a bio-indicator of environmental pollution with heavy metals. *Mellifera* 13:10–20
- Antonovics J, Bradshaw AD, Turner R (1971) Heavy metal tolerance in plants. *Adv Ecol Res* 7:1–85
- Bargańska Ż, Ślebioda M, Namieśnik J (2016) Honey bees and their products: Bioindicators of environmental contamination. *Crit Rev Environ Sci Technol* 46:235–248
- Boonyapookana B, Parkplan P, Techapinyawat S, DeLaune RD, Jugsujinda A (2005) Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). *J Environ Sci Health A* 40:117–137
- Bromenshenk JJ, Carlson S, Simpson J, Thomas J (1985) Pollution monitoring of Puget sound with honey bees. *Science* 227:632–634
- Brown SL, Chaney RL, Hettiarachchi GM (2016) Lead in urban soils: a real or perceived concern for urban agriculture? *J Environ Qual* 45:26–36
- California Office of Environmental Health (2010) Risk assessment – soil and soil gas. OEHHA. <http://oehha.ca.gov/risk/chhsltable.html>. Accessed 09 April 2015
- Citak D, Silici S, Tuzen M, Soylak M (2012) Determination of toxic and essential elements in sunflower honey from Thrace region, Turkey. *Int J Food Sci Technol* 47:107–113
- Cutright T, Gunda N, Kurt F (2010) Simultaneous hyperaccumulation of multiple heavy metals by *Helianthus annuus* grown in a contaminated sandy-loam soil. *Int J Phytorem* 12:562–573
- Devillers J, Pham-Delègue M-H (2002) Honey bees: estimating the environmental impact of chemicals. Taylor and Francis, London
- Di N, Hladun KR, Zhang K, Liu T-X, Trumble JT (2016) Laboratory bioassays on the impact of cadmium, copper and lead on the development and survival of honeybee (*Apis mellifera* L.) larvae and foragers. *Chemosphere* 152:530–538
- Fox J, Weisberg S (2011) An {R} companion to applied regression, Second edn. Sage, Thousand Oaks
- Free JB, Williams IH, Pinsent R, Townshend A, Basi MS, Graham CL (1983) Using foraging honeybees to sample an area for trace-metals. *Environ Int* 9:9–12
- Gall JE, Boyd RS, Rajakaruna N (2015) Transfer of heavy metals through terrestrial food webs: a review. *Environ Monit Assess* 187:201. doi: 10.1007/s10661-015-4436-3
- Gardiner MM, Burkman CE, Prajzner SP (2013) The value of urban vacant land to support arthropod biodiversity and ecosystem services. *Environ Entomol* 42:1123–1136
- Gardiner MM, Prajzner SP, Burkman CE, Albro S, Grewal PS (2014) Vacant land conversion to community gardens: influences on generalist arthropod predators and biocontrol services in urban greenspaces. *Urban Ecosyst* 17:101–122
- Hladun KR, Parker DR, Trumble JT (2011) Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance. *Environ Exp Bot* 74:90–97
- Hladun KR, Parker DR, Tran KD, Trumble JT (2013) Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.) *Environ Pollut* 172:70–75
- Hu LT, Bentler PM (1999) Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct Equ Model* 6:1–55
- Jarup L (2003) Hazards of heavy metal contamination. *Br Med Bull* 68:167–182
- Jennings AA, Petersen EJ (2006) Variability of North American regulatory guidance for heavy metal contamination of residential soil. *J Environ Eng Sci* 5:485–508
- Kastori R, Plesnicar M, Sakac Z, Pankovic D, Arsenijevic-Maksimovic I (1998) Effect of excess lead on sunflower growth and photosynthesis. *J Plant Nutr* 21:75–85
- Kevan PG (1999) Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agric Ecosyst Environ* 74:373–393

- Kohrman H, Chamberlain CP (2014) Heavy metals in produce from urban farms in the San Francisco Bay Area. *Food Addit Contam, Part B* 7:127–134
- 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
- Matteson KC, Ascher JS, Langellotto GA (2008) Bee richness and abundance in New York City urban gardens. *Ann Entomol Soc Am* 101:140–150
- Meindl GA, Ashman T-L (2013) The effects of aluminum and nickel in nectar on the foraging behavior of bumblebees. *Environ Pollut* 177:78–81
- Meindl GA, Ashman T-L (2014) Nickel accumulation by *Streptanthus polygaloides* (Brassicaceae) reduces floral visitation rate. *J Chem Ecol* 40:128–135
- Meindl GA, Ashman T-L (2015) Effects of floral metal accumulation on floral visitor communities: introducing the elemental filter hypothesis. *Am J Bot* 102:379–389
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct Ecol* 18:116–124
- Mogren CL, Trumble JT (2010) The impacts of metals and metalloids on insect behavior. *Entomol Exp Appl* 135:1–17
- Mok H-F, Williamson VG, Grove JR, Burry K, Barker SF, Hamilton AJ (2014) Strawberry fields forever? Urban agriculture in developed countries: a review. *Agron Sustain Dev* 34:21–43
- Parmentier L, Meeus I, Cheroutre L, Mommaerts V, Louwye S, Smagghe G (2014) Commercial bumblebee hives to assess an anthropogenic environment for pollinator support: a case study in the region of Ghent (Belgium). *Environ Monit Assess* 186:2357–2367
- Perugini M, Manera M, Grotta L, Abete MC, Tarasco R, Amorena M (2011) Heavy metal (Hg, Cr, Cd, and Pb) contamination in urban areas and wildlife reserves: honeybees as bioindicators. *Biol Trace Elem Res* 140:170–176
- Pisa LW et al (2015) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res* 22:68–102
- R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Rosseel Y (2012) Lavaan: an R package for structural equation modeling. *J Stat Softw* 48:1–36
- Ruschioni S, Riolo P, Minuz RL, Stefano M, Cannella M, Porrini C, Isidoro N (2013) Biomonitoring with honeybees of heavy metals and pesticides in nature reserves of the Marche region (Italy). *Biol Trace Elem Res* 154:226–233
- Satta A, Verdinelli M, Ruiu L, Buffa F, Salis S, Sassu A, Floris I (2012) Combination of beehive matrices analysis and ant biodiversity to study heavy metal pollution impact in a post-mining area (Sardinia, Italy). *Environ Sci Pollut Res* 19:3977–3988
- Schemske DW, Horvitz CC (1988) Plant animal interactions and fruit production in a neotropical herb: a path analysis. *Ecology* 69:1128–1137
- Schindelin J et al (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682
- Singh A, Prasad SM (2011) Reduction of heavy metal load in food chain: technology assessment. *Rev Environ Sci Biotechnol* 10:199–214
- Stavert JR, Liñán-Cembrano G, Beggs JR, Howlett BG, Pattemore DE, Bartomeus I (2016) Hairiness: the missing link between pollinators and pollination. *PeerJ* 4:e2779
- van der Steen JJM, 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
- Venables WN, Ripley BD (2002) *Modern applied statistics with S*, Fourth edn. Springer, New York