Effects of cadmium soil contamination on pollination services

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Abstract.

Urban agriculture has grown in popularity across many cities throughout the world. Many of these cities have industrial pasts, resulting in soils contaminated with heavy metals such as lead (Pb), cadmium (Cd), and copper (Cu). Heavy metals are known to adversely affect human health, but their effects on the pollinators providing critical pollination services to urban agriculture are largely unknown. The objective of this study was to understand the effects of Cd contamination on bees' pollination services. We predicted that sunflowers grown in Cd-contaminated soil would receive fewer pollination services than those grown in control soil, resulting in lower seed set. To test this, we grew sunflowers in the greenhouse in three soil treatments (uncontaminated potting media, and media contaminated with either 10 ppm or 50 ppm Cd). Once mature, these sunflowers were placed into the field and left open to pollination for six hours on three days. Additional flowers from each soil treatment were either hand pollinated (N = 24) or pollinators excluded (N= 24) to compare seed set to those naturally pollinated. Flowers were maintained in the greenhouse until senescence and their resulting seeds counted. Flowers grown in media containing 50 ppm Cd produced significantly fewer seeds than those grown in uncontaminated media, however the reduced seed set was not due to a loss of pollinator visitation. This suggests that we will see lower sunflower productivity in areas with high levels of Cd, but this reduction is likely not due to a loss of pollinator visitation.

Introduction.

Urban agriculture is a growing practice across cities in the United States (Brown et al. 2016). A type of alternative farming, urban agriculture is based on small-scale local food production in an urban setting (Aerts et al. 2016). Urban farming is especially popular in once densely-populated neighborhoods that are now filled with an abundance of vacant lots created by economic downturn, job loss, and the 2008 foreclosure crisis (Silvia 2010). In addition to giving use back to vacated land, urban agriculture also offers a source of food, ecosystem services and jobs (McClintock, 2010). It can also offer a chance for pollinator conservation by creating an important refuge for pollinators while at the same time benefiting from pollination services.

The increased popularity of urban agriculture offers many benefits, but sustainable food production in cities also has its own challenges. Many of the cities engaged in urban agriculture have an industrial past, and as a result, have soils contaminated with heavy metals such as copper (Cu), cadmium (Cd), and lead (Pb) (Mogren and Trumble 2010). These heavy metals contaminate soil through industrial practices like mining and smelting, and through the historic use of products containing heavy metals, such as leaded fuel and paint (Mogren and Trumble 2010). Excessive accumulation of heavy metals in agricultural soils may affect food quality and safety

(Wong et al. 2002). As a result, the U.S. Environmental Protection Agency has regulations for the concentrations of heavy metals (Jennings & Ma 2007). Thresholds are clear for some heavy metals. For example, urbans farmers must confirm that Pb levels for their arable soil are below the threshold level of 400 ppm (U.S. EPA 2011) before farming can begin on a vacant lot. However, for other metals such as Cd, established thresholds do not exist. Cd is often found in elevated concentrations in urban soils. Increased concentrations of Cd are likely to be found within bumble bee larvae as urbanization increases (Sivakoff et al. *in prep*), and increased concentrations are negatively associated with the diversity and abundance of wild bees (Moroń et al. 2012).

Bees are increasingly used a bioindicators for environmental contaminants (Celli and Maccagnani 2003) such as heavy metals (Devillers and Pham-Delegue 2002). They can be exposed to contamination via multiple avenues, such as accumulations in the nectar and pollen of floral sources (Hladun et al. 2011, Meindl and Ashman 2014). Many studies analyzing the effects of heavy metal contamination on pollinators focus on the impacts heavy metals have on behavior (Meindl and Ashman 2013, Hladun et al., 2013, Meindle and Ashman 2014, Sivakoff and Gardiner 2017), however relatively few focus on the impacts on valuable pollination services.

We assessed the effects of soil Cd contamination on pollination services, specifically the difference in seed set in potted sunflowers grown in either uncontaminated or Cd-contaminated growing media. Seed set is often used to measure the success of pollination services (Willcox et al. 2017). Sunflowers are common in urban agroecosystems and serve as a resource for nectar and pollen for pollinators. We predicted that pollination services would be less for sunflowers grown in Cd-contaminated media when compared to those grown in uncontaminated media, resulting in a lower seed set in contaminated flowers.

Methods.

Soil amendment and plant growth.

To create the Cd-contaminated soil treatments, we thoroughly combined wetted peat-based growing medium (Pro-Mix HP, Premier Tech Horticulture, Québec, Canada) with two different amounts of $CdCl_2$. For the low contamination levels, we mixed the growing medium to a concentration of 10 ppm. We chose the low contamination threshold based on the maximum Cd concentration from soil samples of 32 vacant lots across Cleveland, OH (M.M. Gardiner *unpublished data*). For the high contamination concentration, we mixed the growing medium to a concentration of 50 ppm. The high contamination threshold was based on the range of Cd concentrations in soil near the locations of old smelters. To ensure that all thresholds were correctly met, soil samples from each treatment (n = 6 per soil contamination treatment) were analyzed for Cd by the Basta Soil Lab at Ohio State University. After mixing, we dried the contaminated treatments at 55 °C for one week to remove all moisture. We added our dried, amended soil to 6 in. plastic pots (n = 100 per treatment). We also created control pots, which consisted of uncontaminated media that was prepared in the same manner as the Cd-amended media only without the addition of Cd. Soil testing revealed that the low treatment had substantially higher Cd levels that expected, prompting us to remove this treatment from the experiment. As a result, we re-ran the experiment later in the summer (Round 2; July – October 2017) with only the low (n = 60) and control (n = 60) treatments.

We germinated 'Dwarf Sunspot' sunflowers individually in wetted, peat based growing medium and transplanted seedlings once they produced their first pair of true leaves into pots containing either of the contaminated treatments or the control soil. We added a teaspoon of fertilizer (Osmocote©, 15-9-12, The Scotts Company, Marysville, OH) to each pot. Transplanted seedlings in their respective soil treatments were randomly assigned a location in the greenhouse. A watering pick was inserted into each pot and plants were watered daily to maintain soil moisture. Dwarf Sunspot sunflowers typically produce a single flower head; after their development, but prior to their opening, we covered flower heads in a tightly-woven mesh bag (1 gallon paint strainer) to exclude any pollinators or insects present in the greenhouse.

Experimental trials.

To test the effect of cadmium contamination is soil on pollination services, we placed contaminated and uncontaminated mature sunflowers in a common garden setting for approximately 6 hours on three separate days (*Round 1: 7/18/17, 7/20/17, and 7/26/17; Round 2: 9/29/17, 10/02/17, and 10/07/17*), with new flowers used each day. Prior to the start of the experimental period, each sunflower was assigned to a pollination treatment: naturally pollinated (NP), hand pollinated (HP), and pollinator excluded (PE). Naturally pollinated flowers had their mesh bags removed and were left open to pollinator access during the experimental trial. Hand pollinated flowers served as a positive control and remained bagged while outside. They were pollinated manually once returned to the greenhouse. Pollinator excluded flowers, which served as a negative control, remained bagged while outside and were not manually pollinated. Contaminated and uncontaminated sunflowers from each pollination treatment were arranged in the field in 8 blocks in such a way that there was 1 m between sunflowers within a block and 10 m between neighboring blocks (Figure 1).

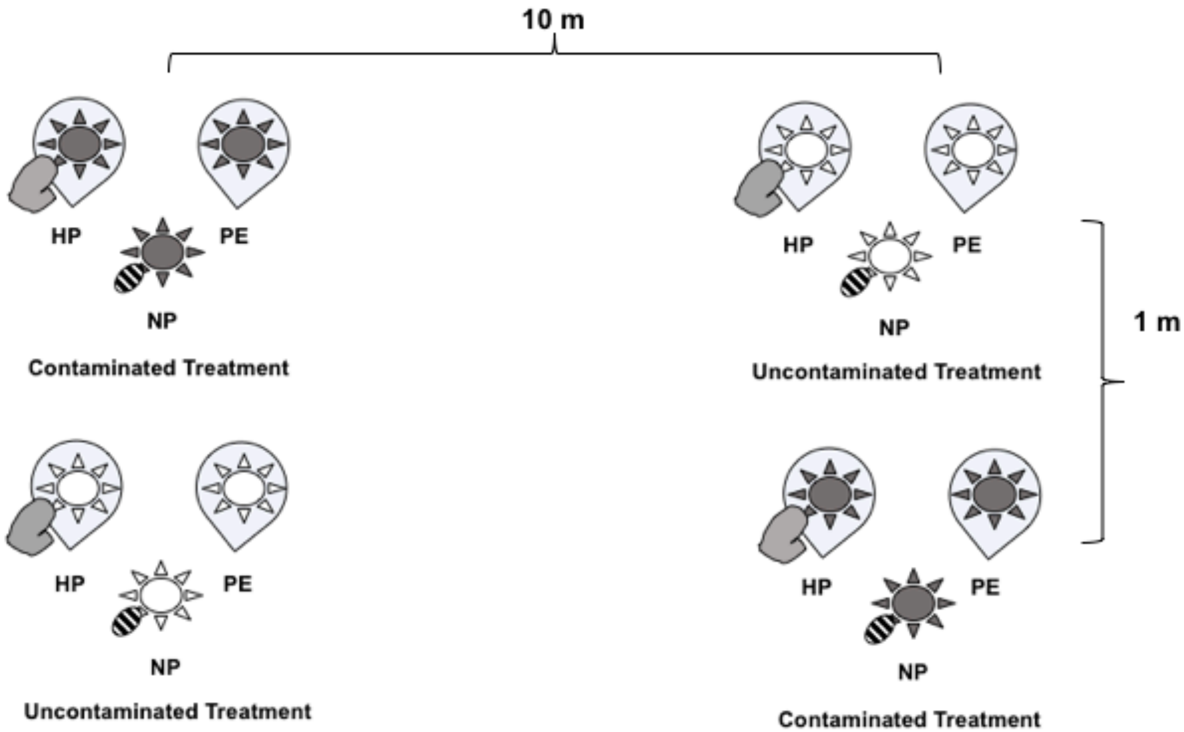

Figure 1. Example of experimental set up. Sets were arranged so that there was 1 m between triplicates in the same set and 10 m between neighboring sets. Neighboring triplicates were from different treatments. Each triplicate contained one flower from each of the pollination treatments: hand pollinated (HP), naturally pollinated (NP), and pollinator excluded (PE).

At the start of the experiment, we carried bagged sunflowers from the greenhouse. Once all flowers were in place, we removed the mesh bags from the flowers assigned to the naturally pollinated treatment. Once we removed the bags, the flower heads were accessible to pollinators for the duration of the experimental period (approximately 09:45 – 15:00). During the experimental period each day, we measured the height of each flower as well as took pictures of each flower head alongside a scale bar to standardize the measurement scale in each image. We later processed these images to quantify the flower head area and the area of open flowers in each flower head using Fiji (Schindelin et al. 2012). At the conclusion of each trial, the naturally pollinated flowers were re-bagged to prevent additional pollination outside of the experimental trial. All flowers were returned to the greenhouse where flowers designated for hand pollination were pollinated using pollen collected from several donor flowers from the corresponding treatments. Flowers were re-bagged to prevent additional pollination. All flowers were maintained in the greenhouse until seed set.

Seed Processing

Flowers were kept in the greenhouse until the heads turned brown, at which point the heads were removed from stems. Flower heads were dried in a 70 °C drying oven for a week. The dried flower heads were broken apart. Developed seeds were separated from chaff using a seed blower, after which the remaining seeds were weighed and the total number calculated.

Statistical analysis

We assessed the effect of soil Cd contamination on pollination services in the statistical platform JMP® 13 (SAS Institute Inc. 2016). We first assessed the difference between the uncontaminated flowers from each round to determine if comparisons across rounds (i.e. between the low and high Cd contamination treatments) could be made. We found statistically significant differences between rounds for all response variables assessed, prompting us to analyze difference between control and high flowers separately from difference between control and lower contamination flowers. Differences in seed set, area of female flowers, area of flower head, and plant height between contaminated (either high or low) and uncontaminated flowers were assessed utilizing one-tailed t-tests, with the assumption that contaminated flowers would have lower seed set and be smaller than uncontaminated flowers. To assess whether pollination treatment affected seed set and whether this difference was affected by contamination, we ran an ANOVA that included the fixed effects of pollination treatment, soil contamination treatment, and their interaction.

Results.

Comparisons between the low contamination and uncontaminated flowers yielded a significant difference in plant height, where low contamination flowers were significantly shorter than uncontaminated (t = 2.12, P = 0.040). However, there was no difference in flower head area (t = -0.32, P = 0.75), area of female flowers (t = 0.57, P = 0.58), and seed set (t = -0.88, P = 0.38). While the difference in seed set between low contamination and uncontaminated flowers was not significant, there were significant differences within the pollination treatments if soil treatment was not taken into consideration. Naturally pollinated flowers set significantly more seeds than pollinator excluded flowers (t = -3.96, P = 0.0005). Hand pollinated flowers also set significantly more seeds than pollinator excluded flowers (t = -2.42, P = 0.046), however there was no significant difference between naturally pollinated and hand pollinated flowers (t = 1.31, P = 0.39; Figure 2A).

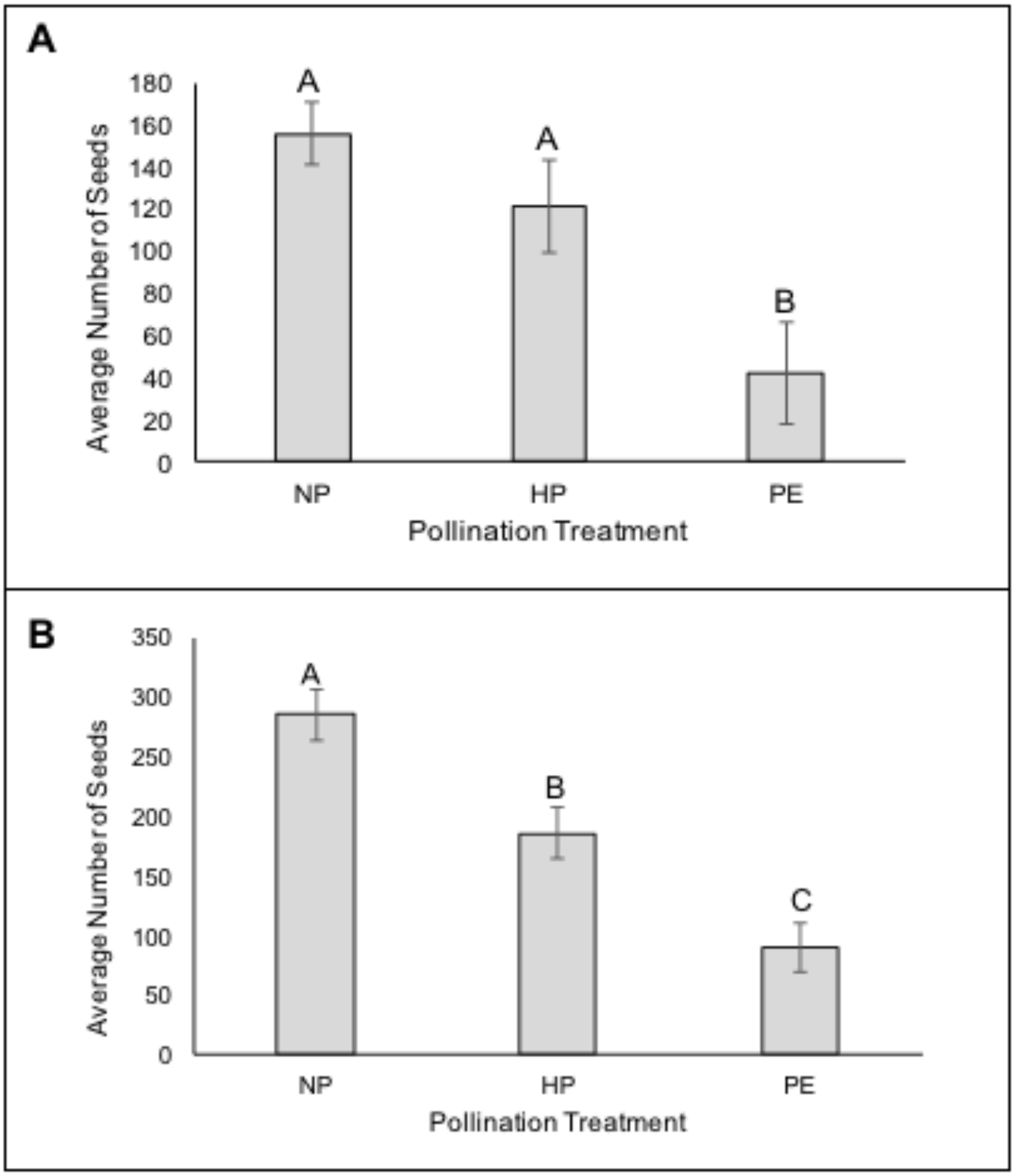

Figure 2. Mean seed set ± SE by pollination treatment for (A) Round 2 and (B) Round 1. From left to right, pollination treatments are naturally pollinated (NP), hand pollinated (HP), and pollinator excluded (PE). Different letters indicate significant differences ($P < 0.05$, Tukey HSD).

Comparisons between the highly contaminated and uncontaminated flowers yielded an insignificant difference in flower head area ($t = 0.13$, $P = 0.89$) and the area of female flowers ($t = 1.45$, $P = 0.92$). However, there were significant differences in flower height, where highly contaminated flowers were significantly shorter than uncontaminated ($t = 2.45$, $P = 0.018$), and seed set, where highly contaminated flowers set significantly fewer seeds than uncontaminated ($t = -1.76$, $P = 0.040$; Figure 3).

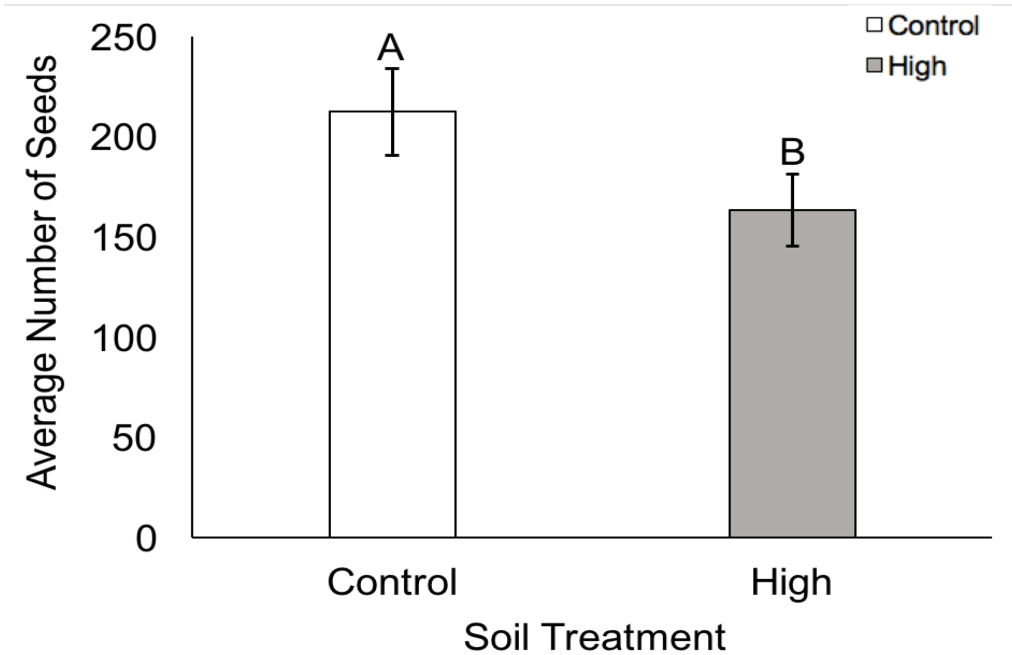

Figure 3. Mean seed set ± SE by soil treatment. Control treatment represented by the white bar. High cadmium contamination treatment represented by the grey bar. Different letters indicate a statistically significant difference between treatments (P <0.0 05) as determined by a one-tailed t-test.

There was no interaction between soil contamination treatment and pollination treatment (F = 0.21, P = 0.81), allowing us to focus on the main effect of pollination treatment, which was significant for the high contamination treatment (F = 21.40, P < 0.001). Naturally pollinated flowers set a significantly higher number of seeds than hand pollinated flowers (t = 3.23, P = 0.004) and pollinator excluded (t = -6.54, P <0.0001). Hand pollinated flowers also set significantly more seeds than the pollinator excluded (t = -3.15, P = 0.0057; Figure 2B).

Discussion.

High concentrations of Cd contamination in soil reduced seed set, however, this reduction was not attributable to a loss in pollination services. We hypothesized that flowers grown in Cd-contaminated soil would receive reduced pollination services, resulting in a lower seed set. Our results instead indicate that flowers grown in contaminated soils received similar pollination services as their uncontaminated counterparts. This suggests that pollinators are not avoiding contaminated plants, which could potentially be because of an inability to discriminate between contaminated and uncontaminated plants. Foragers have been known to have difficulty initially distinguishing between heavy metal contaminated and uncontaminated plants (Meindl and Ashman 2014, Sivakoff and Gardiner 2017). While we did not explicitly measure Cd concentrations in the floral resources available, Cd is known to accumulate in plants (Hladun et al. 2015). Accumulations in the flower head can provide a potential avenue for contamination through the contact and ingestion of contaminated floral resources

(Hladun et al. 2015). Contaminants can bioaccumulate within terrestrial organisms (Gall et al. 2015), and the inability to avoid contaminated resources may cause fatal bioaccumulations in populations.

Naturally pollinated flowers set significantly more seeds than hand pollinated flowers (Figure 2), indicating that pollinators do not appear to be limiting their services. If the flowers were pollinator limited, then we would expect to see a greater seed set in the hand pollinated over the naturally pollinated. The fact that the naturally pollinated flowers set a great number of seeds than the hand pollinated proves that the insects were better pollinators than we and that improvements could be made in our hand pollination methods.

Sunflowers grown in highly contaminated soil were 12% shorter than those grown in uncontaminated soil (25.4 ± 1.00 contaminated vs 28.9 ± 1.02 uncontaminated). Those grown in the low contamination treatment were 8% shorter than those grown in uncontaminated soil (32.7 ± 0.92 contaminated vs 35.4 ± 0.92 uncontaminated), suggesting that Cd influences plant development. Cadmium is readily taken up by plants due to its similarities with the essential micronutrient zinc (Hladun et al. 2015) and impacts plant growth along with several cellular processes (Rana 2015). The reduced seed set that we observed in high Cd plants could also be a result of a physiological interaction of the Cd within the plant.

Our finding that high concentrations of soil Cd contamination affects seed set, but not because of a loss in pollination services can be used to inform the success of pollination within urban areas. In particular, if a site has high levels of Cd, we might expect to see low levels of sunflower productivity, but it will likely not be due to a loss of pollinator visitation. Low concentrations of soil Cd contamination did not have a significant effect on seed set, suggesting that seed set might not be of concern in environmentally realistic levels of Cd. Future studies will examine if Cd contamination is detectable in pollen and nectar resources, as well as what potential influences this may have on bee behavior, fecundity, and survivorship.

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