Exposure to Urban Heavy Metal Contamination Diminishes Bumble Bee Colony Growth

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Abstract

1. As a result of their industrial past, legacy cites often have elevated concentrations of soil heavy metal contamination. Metal pollution can have negative and prolonged ecosystem impacts, and bees that forage in these urban ecosystems are at risk of exposure. Legacy cities are known to support species rich bee communities, which highlights the importance of determining the impact of heavy metal contamination on wild bee health.

2. We examined how oral exposure to concentrations of four heavy metals found within the provisions of urban bees influenced colony growth of *Bombus impatiens* Cresson (Hymenoptera: Apidae), a common species within legacy cities across the eastern United States. Colony weight and brood survivorship were compared among hives fed uncontaminated sucrose solution (hereafter nectar), nectar spiked with one metal (arsenic, cadmium, chromium, or lead), and nectar containing all metals, after 15 or 30 d of exposure within flight tents.

3. Across both exposure periods, we found a significantly higher proportion of dead brood in metal exposed hives. Additionally, colonies fed all four metals had a significantly higher proportion of dead brood than those fed a single metal.

4. *Synthesis and applications.* Our findings illustrate that even low, environmentally relevant concentrations of metals collected by *B. impatiens* in legacy cities can negatively influence bee colony fitness. We highlight the need to identify metal exposure routes for bees in contaminated landscapes to minimize risk and bolster conservation habitat initiative success.

Introduction

Legacy cities have become a focal system for interdisciplinary study of greening benefits (Riley et al. 2018; Riley and Gardiner 2020), including the creation of habitat for bees (Turo and Gardiner 2019). Legacy cities are characterized by long-term economic disinvestment (Martinez-Fernandez et al. 2012; Haase et al. 2014) and vacancy shapes their landscape pattern by creating dynamic mosaics of occupied and abandoned structures, and patches of formerly occupied vacant land (Gardiner et al. 2013; Sampson et al. 2017). Vacant lots provide season long forage for urban bees from urban spontaneous vegetation (Perry et al. 2020), and these habitats can support species rich bee communities. Legacy cities have also become targets for green investments, such as the creation of pocket prairies, urban farms, and rain gardens on vacant land, often with the explicit aim or stacked benefit of supporting pollinator health (Gardiner et al. 2013; Rega-Brodsky and Nilon 2016; Chaffin et al. 2016; Anderson and Minor 2017; Sivakoff et al. 2018). However, the industrial past of legacy cities has resulted in elevated heavy metal contamination within vacant lot soils (Kelly et al. 1996; Davydova 2005), necessitating an investigation into the impacts of heavy metal exposure on bee health.

Bees are known to accumulate heavy metals when foraging within contaminated areas (van der Steen et al. 2012; Sivakoff et al. 2020), demonstrated by the use of the European honey bee as bioindicators of

heavy metal contamination in the landscape (Bromenshenk et al. 1985; Conti and Botrè 2001; Perugini et al. 2011). Similarly, wild caught bumble bees foraging near mining and industrial activity accumulate Pb and Cd in their bodies (Szentgyörgyi et al. 2011), and solitary cavity nesting *Osmia rufa* and *Osmia bicornis* accumulate heavy metals in their provisions when nesting near smelting operations (Moroń et al. 2012, 2014). However, field research has primarily focused on the detection of heavy metals within bees across contamination gradients (van der Steen et al. 2012; Sivakoff et al. 2020) or correlative relationships between soil heavy metals contamination and local bee abundance and species richness (Szentgyörgyi et al. 2011; Moroń et al. 2012). For instance, wild cavity nesting bee species abundance and diversity steadily decreases with increasing heavy metal concentrations in bee- collected pollen (Moroń et al. 2012). While these studies suggest that foraging in heavy metal contaminated sites negatively influences bee fitness, they do not show that these contaminants were the causative agent, as other variation present among sites, such as the concentration or quality of forage, might also explain these patterns. Likewise, laboratory studies have documented that ingestion of heavy metal contaminated food provisions can have lethal and sublethal bee health impacts (Burden et al. 2016a; Di et al. 2016; Polykretis et al. 2016; Rothman et al. 2019). However, the doses of heavy metals examined are rarely informed by environmentally relevant concentrations measured from field-collected provisions.

The legacy of land use is emerging as a critical component of conservation planning, as current ecosystem structure and function are shaped by founding activities decades after they have ceased (Foster et al. 2003). Legacy cities, such as Cleveland, Ohio, USA, were built on iron, steel, chemical and petroleum production (Morton 2002; Wilson and Wouters 2003) but suffered severe economic downturn and mass emigration coinciding with the closure of many of these founding industries (Hartley 2013). Cleveland lost 50% of its population, resulting in over an overabundance of residential infrastructure that was eventually demolished creating 27,000 vacant lots covering 2,000 ha of urban land area. While these reclaimed, converted greenspaces support arthropod biodiversity (Flor et al. 2017, 2020; Sivakoff et al. 2018; Perry et al. 2020; Gardiner et al. 2020; Turo et al. 2021) and supply ecosystem services (Chaffin et al. 2016; Riley et al. 2018), elevated metal concentrations are commonly found within the surface soils of vacant lots (Jennings et al. 2002; Perry et al. 2020). For example, bumble bee workers and brood from hives located within Cleveland were more likely to contain detectable levels of heavy metals than workers from suburban or rural hives (Sivakoff et al. 2020). Alarmingly, negative correlations between the presence of some heavy metals in bees and their provisions, and worker and brood abundance were also found, raising the question if legacy cities with widespread heavy metal contamination are an appropriate context for urban bee conservation investment (Sivakoff et al. 2020).

Our goal was to test the hypothesis that consuming concentrations of heavy metals found within the provisions of urban bees affected colony growth. To test our hypothesis, we utilized environmentally relevant concentrations of heavy metals from provisions collected by bumble bee workers within the city of Cleveland (Sivakoff et al. 2020). We established a bee flight tent study wherein colonies of *B. impatiens* fed uncontaminated provisions were compared against colonies fed heavy metal-spiked nectar with environmentally relevant concentrations of cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), or all combined. We predicted heavy metal-fed colonies would produce fewer brood (eggs, larvae, and

pupae) and weigh less than control colonies at the conclusion of the experiments. We predicted that the reproductive success of colonies would be reduced when exposed to heavy metal contaminated nectar sources.

Methods

Experimental Design: We performed chronic oral exposure toxicity studies on *B. impatiens* colonies using 7 x 2.5 x 2.5 m metal-framed mesh field cages (hereafter tents) at Waterman Agriculture and Natural Resources Laboratory Research Farms at The Ohio State University (Columbus, Ohio, 40.0136222, -83.0405457). We erected twelve tents on a mown grass plot with ground cloth to eliminate weed pressure and secured the tent fabric edges with cinderblocks and mulch to minimize bee escape. We checked tents daily for holes and repaired as necessary. Each tent was equipped with a quart sized modified honey bee gravity feeder containing either a control solution of 50% w/v sucrose: DI water or a treatment sucrose solution (see below), and a pollen feeder containing uncontaminated, commercially produced honey bee collected pollen ground via mortar and pestle (Figure 1). We conducted five tented foraging experiments during the summers of 2018 and 2019, each of which with 12 colonies per experiment, one per tent (N = 60 total colonies deployed). While experiments varied in length and metals tested (Table 1), the general procedure for each experiment is as follows: At the start of the experiment, we randomly assigned a naïve commercial bumble bee colony (Koppert Biological Systems, Howell, MI, USA) containing one queen and approximately 50 workers to each tent. We weighed each colony prior to deployment, placed it on a cinder block to reduce contact with ground moisture, and affixed a corrugated plastic roof to provide shade and rain protection. Bees were allowed to forage in their tent for a set amount of time, and all feeders were checked daily and refilled as necessary to ensure continuous food resources. Twenty-four hours prior to the end of each experiment, the entrance doors of the hives were positioned to allow returning bees to enter the hive, but no bees exit the hive. At the conclusion of the experiment, we collected the colonies, reweighed them, and froze them in a -20° freezer for five days. We then dissected the colonies to determine the number of individuals and the proportion dead in each life stage (egg, larva, pupa, adult, queen). Each life stage was sorted into living and dead based on visual confirmation of color and texture based on previous live colony dissections.

We tested four heavy metals commonly found in urban environments and correlated with lethal and sub-lethal effects on bees: As, Cd, Cr, and Pb (Jennings et al. 2002; Sharma et al. 2015; Sivakoff et al. 2020). As contaminated environments oftentimes have multiple heavy metals, we also evaluated a treatment of all heavy metals combined. Experiments were run for 15 days to allow larvae that arrived with the naïve colony to pupate and emerge and 30 days in order to encompass eggs laid by the queen through pupation and emergence, as it typically takes a bumble bee egg 25 to 37 days to emerge (Heinrich 2002). Since it was not feasible to run all the metal treatments in each round of the experiment, we included control colonies in each round to evaluate consistency and enable comparisons across all rounds. We ran one 15 and one 30-day length experiment for each treatment (Cd, Cr, As, Pb, All heavy metals combined), see table 1. Experiments 1 and 2 consisted of four Cd fed colonies, four Cr fed colonies, and four control colonies, for 15- and 30-day lengths, respectively. Experiments 3 and 4 consisted of four As fed colonies,

four Pb fed colonies, and four control colonies for 15- and 30-day lengths, respectively. For Experiment 5 (all heavy metals combined), we deployed eight treatment and four control colonies on Day 1, collected four random treatment and two control colonies on Day 15, and collected the remaining four treatment and two control colonies on Day 30.

Concentrated test stock heavy metal solutions were prepared prior to experiments. Treatment test heavy metal concentrations were based on the highest metal concentrations found in bumblebee collected provisions from hives deployed in Cleveland, OH (Sivakoff et al. 2020). Test heavy metals included: arsenic (arsenic (III) oxide, As_2O_3; 0.894 ppm), cadmium (cadmium chloride, $CdCl_2$; 0.276 ppm), chromium (chromium (VI) oxide, CrO_3;0.245 ppm), and lead (lead nitrate, $Pb(NO_3)_2$; 0.265 ppm). We prepared treatment and control sucrose solution (hereafter, “nectar”) by creating a 50% w/v sucrose: DI water mixture and shaking until dissolved. Treatment nectar was created by adding stock heavy metal solution to 3.75 liters of prepared nectar and inverting 20 times, and control nectar was created identically to treatment but adding DI water in place of stock heavy metals. The nectar was placed in an inverted quart size jar with pinholes in the lid, placed on spacers, and secured to a cinderblock within a water moat to discourage ants.

Data Analysis

We analyzed data using R version 3.6.3 (R Core Team 2020). Experiment 4 (30 Day As, Pb, and control) was omitted from data analysis because of control colony death, likely a result of weather conditions throughout the experiment outside of bumble bee colony tolerance. In addition, one Cd colony and one control colony from Experiment 1 were omitted from data analysis due to missing data. To make comparisons among experiments, we first evaluated consistency among control colonies across the experiments and found no significant differences in caste abundance or mortality (15 day: F= 4.24, P = 0.07; 30 day: F= 0.01, P = 0.924). To analyze the effect of each heavy metal on the number of individuals in each life stage (eggs, larvae, pupae, adults), we used generalized linear models with the glm function using the MASS package (W.N.Venables *et al.* 2002) and modeled the counts using a negative binomial distribution. The model included treatment and experiment length as fixed effects. To evaluate whether exposure to heavy metals affected the likelihood that an individual was alive at the end of the experiment, we used generalized linear models with a binomial error distribution where the state of an individual at the conclusion of the experiment (alive or dead) was considered as a binary response variable. We used odds ratios with 95% confidence interval to calculate the expected difference in odds that a colony has dead brood given exposure, compared to the odds of dead brood occurring in the absence of exposure. For example, an odds ratio of four would indicate that the odds of a brood within a colony to be dead differ by a factor of four between treatment and control, whereas an odds ratio of one would indicate that the odds of a colony containing dead brood does not differ from the control treatment.

Results

Across all experiments, we found no difference between treatment and control colonies for the total number (live and deceased) in each life stages (eggs, larvae, pupae, adults), or colony weight change (Table 2). For the 15-day experiments, there was a significant difference in the proportion of dead brood between treatment and control colonies; colonies fed a single heavy metal had 3 to 4 times higher likelihood of having dead brood, whereas colonies fed All heavy metals had a 9 times higher likelihood of dead brood (Fig. 2: As: $p < 0.001$, OR= 3.73 (95% C.I: 3.06, 4.57) ; Cd: $p= 0.016$, OR= 3.71 (95% C.I: 2.83, 4.86); Cr: $p< 0.001$, OR= 3.29 (95% C.I: 2.58,4.21); Pb: $p= 0.002$, OR= 3.32 (95% C.I: 2.66, 4.15); All heavy metals: $p< 0.001$, OR= 8.98 (95% C.I: 7.31,11.08)).

For 30-day experiments, we also found a significant difference between control and treatment colonies in the proportion of dead brood. Colonies fed cadmium for 30 days were four times more likely to have dead brood compared to control colonies; chromium fed colonies had 15 times higher likelihood of having dead brood, and colonies fed all four test metals had 4.5 times higher likelihood of containing dead brood (Cd: $p< 0.01$, OR= 3.91 (95% C.I: 2.71, 5.68); Cr: $p< 0.01$, OR= 15.2 (95% C.I: 9.61, 24.81); All heavy metals: $p< 0.01$, OR= 4.56 (95% C.I: 3.14, 6.69).

Discussion

Bumble bee colonies exposed to environmentally relevant concentrations of heavy metals experienced increased brood mortality. This causal link supports the correlations between heavy metal contamination and brood mortality demonstrated in previous studies (Moroń et al. 2014; Sivakoff et al. 2020). The goal of our study was to isolate the impacts of oral heavy metal exposure on bumble bee colony fitness from other factors that may influence colony fitness and survival. These results support the idea that heavy metal contamination is an important determinant of urban bee fitness (Harrison and Winfree 2015; Sánchez-Bayo and Wyckhuys 2019). With burgeoning interest in establishing pollinator habitat within cities and other human modified landscapes – areas that commonly contain heavy metal contaminated soils (Petersen et al. 2006, Perry et al. 2020), our results highlight the critical need to understand and minimize wild bee exposure to metal contamination (Delibes et al. 2001; Tovar-Sánchez et al. 2018).

We predicted that consuming environmentally relevant concentrations of heavy metal contaminated provisions represents a fitness cost for bumblebee colonies through increased brood mortality and decreased colony weight. To examine if urban heavy metal contamination was a true threat to city dwelling bee health, we measured the growth of colonies fed heavy metal concentrations detected in the provisions of bumblebees foraging within the city of Cleveland, Ohio (Sivakoff et al. 2020). Surprisingly, oral exposure to each individual heavy metal resulted in similar brood mortality for all four metals tested, three to four times higher compared to control colonies. Exposure to multiple heavy metals had a synergistic negative effect, as brood mortality was significantly higher in colonies exposed to several heavy metals than colonies exposed to a single metal, up to nine times higher brood mortality compared to control colonies. Certain metals interact to create a synergistic or additive effect, as is seen with the interaction of lead and cadmium, or arsenic and lead in invertebrates (Yoo et al. 2021). Various metals are typically found in contaminated environments; thus, bees are encountering a variety of pollutants per

foraging bout (Szentgyörgyi et al. 2011; Sivakoff et al. 2020). To date, a majority of studies on bees and heavy metals have analyzed the impact of single metal on honey bees, but there is a dearth of controlled, multiple exposure assays demonstrating the importance of understanding toxic interactions between multiple pollutants.

Interestingly, while we found a strong effect of heavy metal contamination on brood survival, we did not find that consuming contaminated provisions negatively influenced worker survivorship. This is similar to what is known about honey bee toxicology, where honey bee larvae raised on heavy metal contaminated pollen expressed fewer detoxification genes and experience higher mortality than female adult honey bees (Di et al. 2016). Bumble bee larvae may have a reduced capacity for heavy metal detoxification compared to adult female bees. Insect detoxification of toxicants, including heavy metals and other xenobiotics, involves diverse strategies that utilize differences in gene expression, enzyme pathways, and epigenetic mechanisms (Merritt and Bewick 2017). Pollinators, in general, appear to have reduced diversity of detoxification pathways compared with phytophagous insects which need to overcome plant defenses, as their genome encodes far fewer genes for detoxification (Liu 2008; Johnson et al. 2012). Additionally, larvae and males typically express fewer detoxification genes than adult females, as is seen with *Bombus huntii* (Xu et al. 2013), which may explain high brood mortality when exposed to heavy metals. Without robust detoxification capacity, bee brood are particularly sensitive to toxicants which may lead to altered colony structure and increased stress on the colony.

While we did not document lethal effects of heavy metals on workers, sublethal or behavioral changes from metal exposure may also have contributed to the reduced brood survivorship within heavy metal exposed colonies. For instance, workers that consume contaminated provisions may have reduced brood care efficiency or nest thermoregulation. For example, exposure to neonicotinoid pesticides alter bumble bee nurse and caretaking behaviors, including thermoregulation and social dynamics, that negatively impacts brood development (Crall et al. 2018). While heavy metals may have different modes of action, it is possible that similar adverse behavioral modifications may result. Furthermore, metals have sublethal negative impacts on bee memory. Selenium intoxication impairs honey bee memory and long term recall abilities, compromising foraging efficiency and return navigation, leading to fewer returning foragers and less available work force (Burden et al. 2016b). Additional to brood starvation, a reduction in available food in bumble bee colonies causes a shift from end of season queen production to worker production, altering reproductive output for the following year (dos Santos et al. 2016). These colony level impacts may cause drastic population level trends in following years and alter bee community composition, abundance, and dominance, but more work is needed in this area to fully understand the implications of metal exposure on bee populations.

Conclusion

By isolating the effects of heavy metal pollution within a semi-field experiment, we illustrate that that foraging within legacy cities could present a health risk to *B. impatiens*. To the best of our knowledge, this paper is the first to isolate the effects of environmentally derived concentrations of relevant heavy metals

on bumblebee colony growth. From previous work by Sivakoff et al. (2020), we know that provisions collected from the legacy city of Cleveland, Ohio, contain detectable concentrations of heavy metals, representing a key exposure route for urban bees. However, to offer any mitigation plan to reduce such exposure requires the determination of bee exposure routes as a next critical step. For instance, the contamination of collected provisions could result from plant uptake of soil heavy metals (Xun et al. 2017) or the deposition of heavy metal contaminated dust on flower heads and planar plant surfaces (Kim et al. 2015; Gajbhiye et al. 2016). Certain plants have the ability to uptake and sequestration of particular heavy metals (Rascio and Navari-Izzo 2011) when growing in metal contaminated media (soil or water) by absorbing metals through the roots and concentrating high levels of metals in tissues (Reeves et al. 2018) and in some instances floral rewards (Meindl et al. 2014; Xun et al. 2017). Importantly, urban spontaneous vegetation species, known to be important urban bee forage (Robinson and Lundholm 2012; Sivakoff et al. 2018; Turo et al. 2021), have not been a major focus for heavy metal toxicological studies. Further, additional contamination routes such as drinking heavy metal contaminated water from puddles or runoff (Sansalone et al. 1996), direct dermal contact with contaminated dust deposited on surfaces (Christoforidis and Stamatis 2009), or other unidentified exposure routes (Zioga et al. 2020) could add to the heavy metal burden faced by urban bees. Once identified, certain practices can help reduce exposure risk, such as mowing highly contaminated habitats prior to bloom, reducing open soil, or prioritizing habitat establishment in less contaminated areas. Unfortunately, heavy metal contamination is not limited to urban landscapes, but rather is present worldwide in varying degrees (Su et al. 2014). Metals are continuously added to the landscape through industrial, agricultural, and domestic activities (Wuana and Okieimen 2011), as well as catastrophic point source events (2019, 2020). Because of this, metal pollution is a persistent and wide spread threat to habitat integrity and ecosystem health and should be a factor when developing bee conservation strategies.

Declarations

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Author Contributions:

S.B.S., M.M.G., and F.S.S conceived the ideas and designed methodology; S.B.S collected the data; S.B.S and F.S.S. analyzed the data and interpreted the results; S.B.S led the writing of the manuscript with revisions from M.M.G and F.S.S; all authors gave final approval for publication.

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Tables

Table 1. Start and end dates, experiment duration, and the treatments and replicates of cage studies.

	Start Date	End Date	Exp length (days)	Treatment 1, # replicates	Treatment 2, # replicates	Control replicates
Exp 1	26/06/2018	12/07/2018	15	Cd (n=4)	Cr (n=4)	(n=4)
Exp 2	24/07/2018	23/08/2018	30	Cd (n=4)	Cr (n=4)	(n=4)
Exp 3	22/05/2019	05/06/2019	15	As (n=4)	Pb (n=4)	(n=4)
Exp 4*	28/06/2019	28/07/2019	30	As (n=4)	Pb (n=4)	(n=4)
Exp 5	06/08/2019	20/08/2019	15	All HM (n=4)		(n=2)
	06/08/2019	03/09/2019	30	All HM (n=4)		(n=2)

* Exp 4 omitted from data analysis due to control colony death

Table 2. The number of larvae, pupae, adults and eggs produced and change in colony weight recorded after 15 or 30 d (Mean +/- SEM) within control or heavy metal treatments (As, Cd, Cr, Pb, All).

	Exp Length	Larvae	Pupae	Adults	Eggs	Colony weight change (g)
Control	15	83 ± 21 (9)	53 ± 40 (9)	245 ± 99 (9)	38 ± 36 (9)	0.06 ± 0.02 (9)
Arsenic	15	87 ± 16 (4)	75 ± 65 (4)	264 ± 33 (4)	10 ± 3 (4)**	0.05 ± 0.05 (4)
Cadmium	15	65 ± 47 (3)	29 ± 13 (3)	321 ± 96 (3)*	43 ± 7 (3)	0.07 ± 0.02 (3)
Chromium	15	49 ± 51 (4)	40 ± 16 (4)	257 ± 46 (4)	55 ± 35 (4)	0.08 ± 0.03 (4)
Lead	15	71 ± 19 (4)	45 ± 28 (4)	287 ± 22 (4)	10 ± 8 (4)*	0.08 ± 0.05 (4)
All	15	154 ± 83 (4)*	40 ± 6 (4)	246 ± 20 (4)	40 ± 18 (4)	0.05 ± 0.05 (4)
Control	30	23 ± 30 (6)	15 ± 6 (6)	201 ± 51 (6)	25 ± 16 (6)	0.15 ± 0.06 (6)
Cadmium	30	49 ± 39 (4).	21 ± 7 (4)	153 ± 62 (4)	26 ± 18 (4)	0.17 ± 0.05 (4)
Chromium	30	20 ± 14 (4)	44 ± 32 (4)*	226 ± 35 (4)	14 ± 20 (4)	0.13 ± 0.06 (4)
All	30	50 ± 34 (4).	18 ± 19 (4)	210 ± 71 (4)	11 ± 9 (4).	0.17 ± 0.02 (4)

[a] Data are presented as mean ± standard error (replicates)

- Significant difference compared with control using negative binomial test (. = 0, * = 0.0, ** = 0.00)

Figures

Figure 1

We performed chronic oral exposure toxicity studies on B. impatiens colonies using 7 x 2.5 x 2.5 m metal-framed mesh field cages (hereafter tents) at Waterman Agriculture and Natural Resources Laboratory Research Farms at The Ohio State University (Columbus, Ohio, 40.0136222, -83.0405457). We erected twelve tents on a mown grass plot with ground cloth to eliminate weed pressure and secured the tent fabric edges with cinderblocks and mulch to minimize bee escape. We checked tents daily for holes and

repaired as necessary. Each tent was equipped with a quart sized modified honey bee gravity feeder containing either a control solution of 50% w/v sucrose: DI water or a treatment sucrose solution (see below), and a pollen feeder containing uncontaminated, commercially produced honey bee collected pollen ground via mortar and pestle.

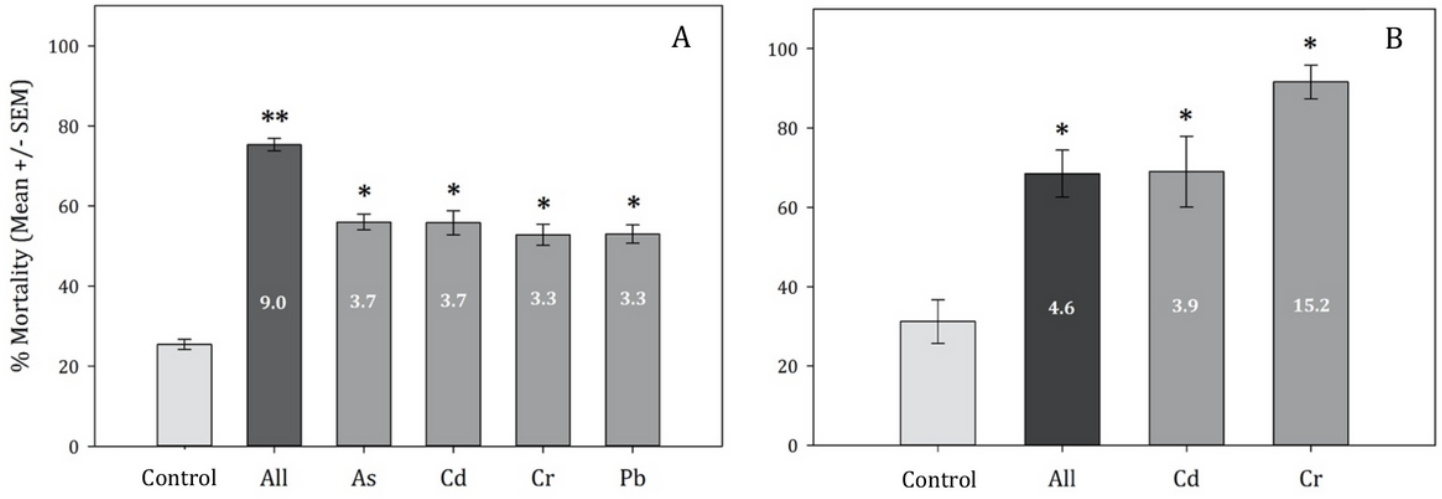

Figure 2

Across all experiments, we found no difference between treatment and control colonies for the total number (live and deceased) in each life stages (eggs, larvae, pupae, adults), or colony weight change (Table 2). For the 15-day experiments, there was a significant difference in the proportion of dead brood between treatment and control colonies; colonies fed a single heavy metal had 3 to 4 times higher likelihood of having dead brood, whereas colonies fed All heavy metals had a 9 times higher likelihood of dead brood (Fig. 2: As: $p < 0.001$, OR= 3.73 (95% C.I: 3.06, 4.57) ; Cd: $p= 0.016$, OR= 3.71 (95% C.I: 2.83, 4.86); Cr: $p< 0.001$, OR= 3.29 (95% C.I: 2.58,4.21); Pb: $p= 0.002$, OR= 3.32 (95% C.I: 2.66, 4.15); All heavy metals: $p< 0.001$, OR= 8.98 (95% C.I: 7.31,11.08)).