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Exposure and Effects of Environmental Stressors to Bees

METAL CONTAMINANT ACCUMULATION IN THE HIVE: CONSEQUENCES FOR WHOLE-COLONY HEALTH AND BROOD PRODUCTION IN THE HONEY BEE (*APIS MELLIFERA* L.)

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Abstract: Metal pollution has been increasing rapidly over the past century, and at the same time, the human population has continued to rise and produce contaminants that may negatively impact pollinators. Honey bees (*Apis mellifera* L.) forage over large areas and can collect contaminants from the environment. The primary objective of the present study was to determine whether the metal contaminants cadmium (Cd), copper (Cu), lead (Pb), and selenium (Se) can have a detrimental effect on whole-colony health in the managed pollinator *A. mellifera*. The authors isolated small nucleus colonies under large cages and fed them an exclusive diet of sugar syrup and pollen patty spiked with Cd, Cu, Pb, and Se or a control (no additional metal). Treatment levels were based on concentrations in honey and pollen from contaminated hives around the world. They measured whole-colony health including wax, honey, and brood production; colony weight; brood survival; and metal accumulation in various life stages. Colonies treated with Cd or Cu contained more dead pupae within capped cells compared with control, and Se-treated colonies had lower total worker weights compared to control. Lead had a minimal effect on colony performance, although many members of the hive accumulated significant quantities of the metal. By examining the honey bee as a social organism through whole-colony assessments of toxicity, the authors found that the distribution of toxicants throughout the colony varied from metal to metal, some caste members were more susceptible to certain metals, and the colony's ability to grow over time may have been reduced in the presence of Se. Apiaries residing near metal-contaminated areas may be at risk and can suffer changes in colony dynamics and survival. *Environ Toxicol Chem* 2016;35:322–329. © 2015 SETAC

Keywords: Cadmium Copper Pollinator Pollutant Selenium

INTRODUCTION

Metal and metalloid pollutants have been increasing rapidly over the past century because of anthropogenic emissions into the environment [1,2]. At the same time, the human population has continued to rise, and these pollutants may negatively impact the pollinators that are needed to produce the food that sustains this population. Over the past 50 yr, the total cultivated area worldwide has increased 33% [3] in an effort to compensate for decreased yield of pollinator-dependent crops [4]. In addition, the number of new crops being developed that rely on pollination is increasing rapidly [5]. Scientific studies are needed to determine which pollutants will be the most important to regulate to protect pollinators that provide critical pollination services for high crop yield.

Metal pollutants are discharged into the air, water, and soil through activities such as mining [6], agriculture [7], coal burning [8], hydraulic fracturing to extract gas and oil [9], and industrial and municipal waste production [10]. Agroecosystems fertilized with manures and biosolids can become contaminated with metals [11], and repeated fungicide application can cause the buildup of metals such as copper (Cu) in perennial fruit or nut crops [12], particularly in the Central Valley of California [13]. Point sources of soil pollution from mining activities can create mine spoils (disposal of metal-rich excavation wastes) or mine tailings from acid ores, thus releasing high concentrations of metals that can alter the surrounding plant community, leaving behind only the most tolerant species [14,15]. In a recent study, Morón et al. [16] found increased levels of cadmium (Cd), lead (Pb), and zinc in the pollen provisions of cavity-nesting bees along 2 gradients of heavy metal pollution at increasing distances from 2 smelters. In addition to these high levels of pollutants, cavity-nesting bees were less diverse and less abundant closer to the smelters. The megachilid bee *Osmia rufa* created fewer brood cells and showed greater mortality along these same pollution gradients [17]. Together, these data suggest that metals can affect insect pollinators other than honey bees.

Varying amounts of metal pollutants have been found in honey bee hives and their products, particularly when located in close proximity to urban or industrial areas (Table 1). Honey bees forage over very large areas and bring plant materials (nectar, pollen, and propolis) back to their hives; thus, they may collect significant amounts of toxic contaminants, making them ideal samplers of the environment [18–20]. Indeed, most research regarding pollutants and bees focuses on their use as bioindicators. Beyond studies of honey bees as bioindicators, there are very limited toxicological data on the effects of metal or metalloid contaminants on pollinators. Some research is available on the effects of metals on honey bee behavior [21,22] and survival [23], but colony-level impact has been rarely, if ever, reported.

Exposure to Cd, Cu, Pb, and selenium (Se) may be detrimental for honey bees at the individual level. Mortality can occur during both larval and adult life stages, and toxicity may manifest itself as both lethal and sublethal effects. Collectively, these results suggest that honey bee colonies would be affected by a metal-contaminated environment.

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Table 1. Examples of metal concentrations for cadmium, copper, lead, and selenium in plants and bee products worldwide

Contaminant	Source	Location	Concentrations ($mg\,kg^{-1}$)	Reference
Cadmium				
	Apis mellifera			
	Workers	Urban and industrial areas, Belgium	0.06–0.10	[54]
	Adult foragers	City center/near highway, Italy	2.87–4.23	[55]
	Adult foragers	Industrial sites, Finland	0.05–1.2	[56]
	Adult foragers	Industrial locality, Czech Republic	0.74–1.75	[34]
	Adult foragers	Polluted areas, Italy	0.05–0.06	[57]
	Adult foragers	Industrialized region, Poland	0.39–0.81	[36]
	Honey	Urban location, Turkey	0.32	[58]
	Honey	Industrial location, Romania	0.0167	[59]
	Honey	Agricultural and industrial areas, Egypt	0.1–0.41	[35]
	Plants			
	Clover flower	Agricultural and industrial regions, Egypt	0.41	[35]
	Raphanus sativus flowers	Greenhouse	13	[60]
Copper				
	A. mellifera			
	Adult foragers	Industrial sites, Finland	14–27	[56]
	Adult foragers	Industrial locality, Czech Republic	31.89–37.68	[34]
	Adult foragers	Industrialized region, Poland	20.2–25.5	[36]
	Honey	Field, Nigeria	25	[61]
	Honey	Field, Turkey	0.2	[62]
	Honey	Agricultural and industrial areas, Egypt	2.3–11	[35]
	Honey	Industrialized region, Poland	0.01–23.5	[36]
	Plants			
	Clover flower	Agricultural and industrial regions, Egypt	51	[35]
	R. sativus flowers	Greenhouse	32	[60]
Lead				
	A. mellifera			
	Workers	Urban and industrial areas, Belgium	0.33–0.41	[54]
	Adult foragers	Industrialized region, Poland	1.46–2.32	[36]
	Honey	Industrial areas, Romania	0.19–0.20	[59]
	Honey	Agricultural and industrial regions, Egypt	1	[35]
	Plants			
	Clover flower	Agricultural and industrial regions, Egypt	2.9	[35]
	Pollen	Urban area, Italy	0.272	[55]
	R. sativus flowers	Greenhouse	1.16	[60]
Selenium				
	A. mellifera			
	Adult foragers	Industrialized region, Poland	1.66–11.04	[63]
	Honey	Industrialized region, Poland	0.11–0.83	[36]
	Plants			
	R. sativus flowers	Field	25	[24]
	R. sativus pollen from corbicula	Field	5.6–2830.2	[24]
	Brassica juncea nectar	Greenhouse	110	[26]
	Stanleya pinnata nectar	Greenhouse	150	[26]

Although some honey bee products may contain relatively low levels of metals (Table 1), sublethal effects such as altered development and learning could reasonably be expected to cause negative consequences for the colony as a whole.

The primary objective of the present study was to determine whether Cd, Cu, Pb, or Se contaminants can have a detrimental effect on whole-colony health in the managed pollinator *Apis mellifera*. By examining the honey bee as a social organism through whole-colony assessments of toxicity, we can investigate the distribution of toxicants throughout the colony, the differences in toxicity among castes, and the colony's ability to grow and function in the presence of toxicants.

MATERIALS AND METHODS

Colony treatments

Packages consisting of 1.36 kg of *A. mellifera ligustica* workers with queens were purchased from C.F. Koehnen and Sons and installed into colony boxes in April 2014 (9 colonies total) and 2015 (10 colonies total). Small nucleus colonies were randomly assigned to a metal treatment, and each colony was a unit of replication. Workers from packages were weighed pretreatment and used as a covariate in the colony weight analysis. Each colony consisted of a 14-L corrugated cardboard colony box containing 4 new, combless standard 23.18-cm black plastic frames and a 3.78-L plastic syrup feeder (Mann Lake).

From the day of installation, colonies were maintained in the experiment for 60 d. Bees were fed a diet of 50% granulated sugar syrup, which was added to the in-hive feeders weekly. A pollen patty was added twice to each colony, at the beginning and middle of the experiment, as a protein source for a total of 454 g of pollen patties added to each colony. Pollen patties consisted of 150.7 g of Bee Pro Pollen Substitute (Mann Lake), 150.7 g of granulated sugar, and 82.84 g of fructose dissolved in 190 mL water. Each patty was weighed to 227 g, wrapped with wax paper, and frozen at –20 °C until use. Colonies were randomly assigned a treatment and placed under a 3 m × 3 m

cage with mesh screen walls (International E-Z Up) in a field 3 m apart from each other, to prevent bees from foraging on other resources besides the food provided.

Metal treatments consisted of sugar syrup and pollen patties dosed with metals to mimic honey and flower concentrations collected from hives in contaminated environments located worldwide (Table 1). We averaged the concentrations in honey and flowers to determine a realistic level to apply to the nucleus colonies. Flower concentrations were not available from Se-contaminated sites. Therefore, pollen patties were conservatively based on the lower range of concentrations from pollen collected in a manipulative field experiment [24]. Cadmium was added as cadmium chloride ($CdCl_2$; Fisher Scientific), Cu was added as cupric chloride dihydrate ($CuCl_2 \times 2H_2O$; Fisher Scientific), Pb was added as lead chloride ($PbCl_2$; Acros Organics), and Se was added as sodium selenate (Na_2SeO_4; Sigma-Aldrich). Metal concentrations in sugar syrup were as follows: control, 0 mg metal/kg; Cd, 0.24 mg/kg; Cu, 25 mg/kg; Pb, 0.5 mg/kg; and Se, 0.6 mg/kg. Metal concentrations in pollen patties were as follows: control, 0 mg metal/kg pollen patty; Cd, 0.46 mg/kg; Cu, 50 mg/kg; Pb, 1.6 mg/kg; and Se, 6 mg/kg. A water source was also provided to each colony in a 3-L plastic dish and replenished twice per week. The water contained 0.08 ± 0.04 mg Cd/L, 1.90 ± 0.97 mg Cu/L, 0.24 ± 0.12 mg Pb/L, and 0.05 ± 0.02 mg Se/L ($n = 4$–6).

Fitness, behavior, and accumulation responses

Direct surface measurements of the frames within each colony were taken 3 times during the experiment using protocols similar to those suggested in Delaplane et al. [25]. Several measures of colony strength were estimated using a wire grid that covered the entire frame with a surface area of 812.8 cm^2. Each grid contained 32 squares measuring 25.4 cm^2 per square. We measured the surface area of wax comb, honey stores, and brood. Photographs of both sides of each frame were taken using a digital single-lens reflex camera on a tripod. The grid was placed over each frame every 14 d. Photos were downloaded off the camera as uncompressed high-quality jpeg files. Adobe Photoshop was used to outline the surface area of wax, honey, and brood; and pixels of each area were recorded and converted to square centimeters. Whole-colony weights were measured 3 times during the experiment using a Weiheng digital scale (Guangzhou Weiheng Electronics). Forager activity was also measured 35 d into the experiment. The total numbers of foragers found within the screened cage and outside each colony were determined.

At the end of the experiment, total worker weight, queen fresh weight, total number of capped brood, and number of dead pupae within capped cells were quantified. Queen and worker weights were measured using a microbalance (weighing to 0.0001 g, model HT224; Shinko Denshi). Total pollen patty consumed was measured by weighing the remaining patty materials and subtracting this value from the starting weight of the pollen patty added.

Insect tissues and honey were collected 3 times during the experiment. We collected approximately 0.327 g of honey directly from the frame by pipetting out of the cell into an Eppendorf tube. Samples were weighed immediately before digestion.

Metal analyses

Five live foragers (found clinging to the screen walls), dead foragers (near the hive entrance), workers (from within the hive), pupae, and honey were collected and frozen in a –60 °C freezer (Fisher Scientific) for metal analyses. Sugar syrup and pollen patty samples were collected to confirm treatment concentrations and frozen at –60 °C. Tissues were freeze-dried (Labconco) at –40 °C and –25 psi for at least 3 d. Honey was also collected and frozen but digested as fresh material. Up to 5 individual insects were pooled within a replicate to create a sufficient tissue weight for analysis. Insect tissues and honey samples were weighed using a microbalance prior to microwave digestion. Insect tissues and honey were then microwave-digested in 110-mL Teflon-lined vessels containing 5 mL concentrated HNO_3 [26]. The vessels were heated for 20 min using a 570-W microwave oven (CEM). Insect tissue and honey digestates were then diluted in a 6 M HCl matrix, heated in a 90 °C water bath for 20 min, and analyzed using inductively coupled plasma optical emission spectroscopy (PerkinElmer). Samples were run in duplicate, with Cd, Cu, Pb, and Se added as internal standards to determine precision and recovery. National Institute of Standards and Technology (US Department of Commerce) standard reference material 1566B (oyster tissue) was used to verify recovery of Cd, Cu, Pb, and Se from a similar biological matrix. All duplicate samples had a precision of 2.7% to 8.6% accuracy ($n = 7$). Metal recoveries for National Institute of Standards and Technology reference material 1566B were as follows: Cd = 90.3%, Cu = 94.5%, Pb = 85.1%, Se = 100.5%.

Statistical analysis

Each colony was a unit of replication. Colony replicates were as follows: control, $n = 5$; Cd, $n = 4$; Cu, $n = 4$; Pb, $n = 4$; and Se, $n = 2$. Each metal (control, Cd, Cu, Pb, Se) was a separate treatment. Originally we had 3 colonies with Se treatment; but the queen absconded from 1 of the colonies early on, and we could not obtain data from that hive henceforth. Colony was nested within treatment as a random effect. Colonies were sampled at 3 sample dates in 2014 and 3 sample dates in 2015. Each metal was analyzed separately. Metal treatment (control, Cd, Cu, Pb, or Se), sample date, and the interaction of sample date and treatment were the independent variables. The fitness responses analyzed were wax comb area, honey area, brood area, and whole-colony weight. These data were analyzed using analysis of variance (ANOVA; PROC GLM; SAS 9.2; SAS Institute). Forager activity, queen and worker weights, pollen patty weight consumed, capped brood, and dead pupae were analyzed using ANOVA (PROC GLM, SAS 9.2; SAS Institute) with type III sum of squares; the independent variable was metal treatment, and each metal was analyzed separately. Brood surface areas for Cd, Cu, and Pb were log-transformed to meet assumptions of normality. Accumulation of Cu in honey was log-transformed. Accumulation of Pb in pupae and queens as well as numbers of capped adult and live pupae brood were log-transformed. Mean separations for each tissue's average metal concentration in Cd-treated, Cu-treated, Pb-treated, or Se-treated colonies were compared with the average metal concentration found in control-treated colonies using post hoc Dunnett's test ($\alpha = 0.05$).

RESULTS

Fitness and behavior during experiment

Cadmium treatments significantly affected honey production in terms of capped cells ($F_{1,5} = 14.52$, $p < 0.02$) but not wax or brood area ($p > 0.24$; Figure 1). Colonies treated with Cd produced significantly more honey. Copper and Pb did not significantly impact wax, honey, or brood surface area. Treatment with Se ($F_{1,4} = 23.82$, $p < 0.01$) significantly reduced

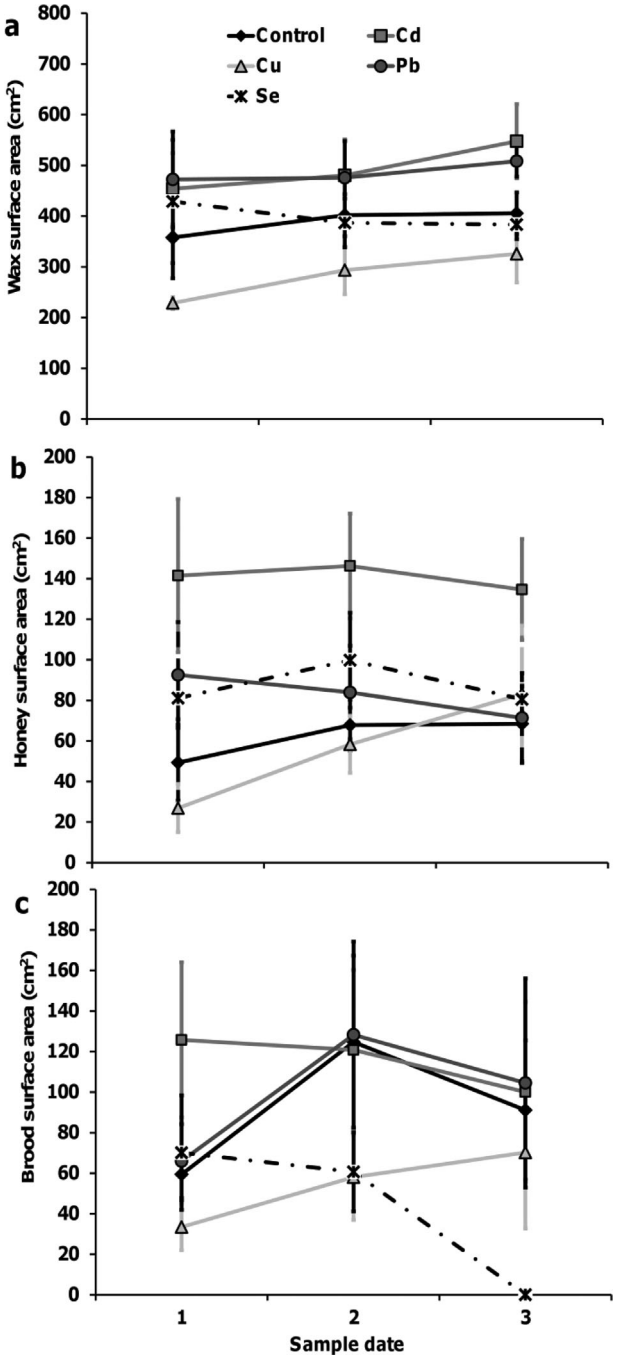

Figure 1. Colony health measurements on frames from honey bee colonies treated with control ($n = 5$), Cd ($n = 4$), Cu ($n = 4$), Pb ($n = 4$), or Se ($n = 2$). Graphs show the amount of surface area measured of (**a**) wax, (**b**) honey, and (**c**) brood over 3 sample dates during the 60-d period of exposure to the metal treatments.

brood surface area (Figure 1c). Time did not have a significant effect on wax, honey, or brood surface area for all treatments ($p > 0.16$ for all). Metal treatments did not affect whole-colony weight ($p > 0.10$ for all; average weight for control, 2.05 ± 0.14 kg; Cd, 1.91 ± 0.20 kg; Cu, 1.72 ± 0.12 kg; Pb, 1.74 ± 0.10 kg; Se, 2.04 ± 0.19 kg) or forager activity during the experiment ($p > 0.15$ for all; average number of foragers for control, 77 ± 26. Cd, 231 ± 147. Cu, 336 ± 110. Pb, 98 ± 62. Se, 58 ± 55; $p = 0.28$). Time significantly increased whole-colony weight for all treatments ($F_{3,3} > 117.72$, $p < 0.001$).

Fitness at end of experiment

In Cd-treated colonies, there were more capped brood ($F_{4,8} = 14.43$, $p < 0.02$) and dead pupae found inside the capped cells ($F_{4,8} = 14.43$, $p < 0.02$; Table 2) at the conclusion of the experiments. Copper-treated colonies showed a similar effect; there were more capped brood ($F_{4,8} = 14.43$, $p < 0.02$) and dead pupae ($F_{4,8} = 14.43$, $p < 0.02$) remaining at the end of the experiment compared with control colonies. Capped cells from Cd-treated colonies contained as high as 78% dead brood, and Cu-treated colonies had up to 89% dead brood. However, despite having more dead pupae, Cd and Cu treatments had similar total worker weight compared with control. Only the Se treatment had a significant effect on reducing the total worker weight ($F_{1,5} = 10.51$, $p < 0.03$; Table 2). Colonies treated with Se also had very few capped cells and no pupae. Cadmium and Cu had no effect on queen weight or total worker weight ($p > 0.24$). Lead treatments did not have a significant effect on any of the fitness endpoints measured ($p > 0.053$).

Colonies fed Cu or Se treatments consumed approximately 35% and 42% less pollen patty compared to the controls, respectively ($F_{4,8} = 14.43$, $p < 0.02$). Cadmium or Pb treatment did not affect the consumption of pollen patties.

Metal accumulation

Cadmium-treated colonies significantly accumulated the metal in queens ($F_{1,6} = 8.76$, $p < 0.03$), workers ($F_{1,6} = 9.35$, $p < 0.03$), dead foragers ($F_{1,6} = 6.71$, $p < 0.05$), and honey ($F_{1,6} = 12.35$, $p < 0.02$) compared with controls. Only live foragers and pupae did not accumulate significant quantities of Cd (Figure 2).

Copper treatments caused significant accumulation in workers ($F_{1,6} = 682.51$, $p < 0.001$), dead foragers ($F_{1,6} = 23.54$, $p < 0.01$), live foragers ($F_{1,6} = 198.10$, $p < 0.001$), pupae ($F_{1,5} = 20.20$, $p < 0.01$), and honey ($F_{1,6} = 11.24$, $p < 0.02$). Queens from Cu-treated colonies did not accumulate significant quantities of the metal ($p > 0.06$; Figure 2b).

Colonies fed Pb treatments significantly accumulated the metal in queens ($F_{1,4} = 8.75$, $p < 0.05$), workers ($F_{1,6} = 9.80$, $p < 0.02$), and live foragers ($F_{1,5} = 9.30$, $p < 0.03$). Dead

Table 2. Analysis of variance showing the effects of 4 metal treatments (Cd, Cu, Pb, and Se) on the fitness of *Apis mellifera* (Hymenoptera:Apidae) nucleus colonies[a]

	Total capped cells	Live pupae	Dead pupae	Adults	Total worker weight (g)	Queen weight (g)
Control	9 ± 5 (5)	6 ± 3 (5)	3 ± 2 (5)	0 ± 0 (5)	184.48 ± 31.67 (5)	0.07 ± 0.01 (4)
Cd	230 ± 34 (4)*	40 ± 26 (4)	180 ± 47 (4)*	10 ± 8 (4)	272.18 ± 65.13 (4)	0.06 ± 0.00 (4)
Cu	105 ± 44 (4)*	6 ± 6 (4)	108 ± 54 (3)*	11 ± 7 (4)	273.23 ± 101.36 (4)	0.06 ± 0.00 (3)
Pb	180 ± 97 (3)	40 ± 40 (3)	82 ± 64 (3)	59 ± 59 (3)	152.00 ± 44.15 (4)	0.06 ± 0.01 (2)
Se	3 ± 3 (2)	0 ± 0 (2)	0 ± 0 (2)	3 ± 3 (2)	12.7 ± 0.2 (2)*	0.06 ± 0.01 (2)

[a]Data are presented as mean ± standard error (n).
*Significant difference compared with control using a post hoc Dunnett's test.

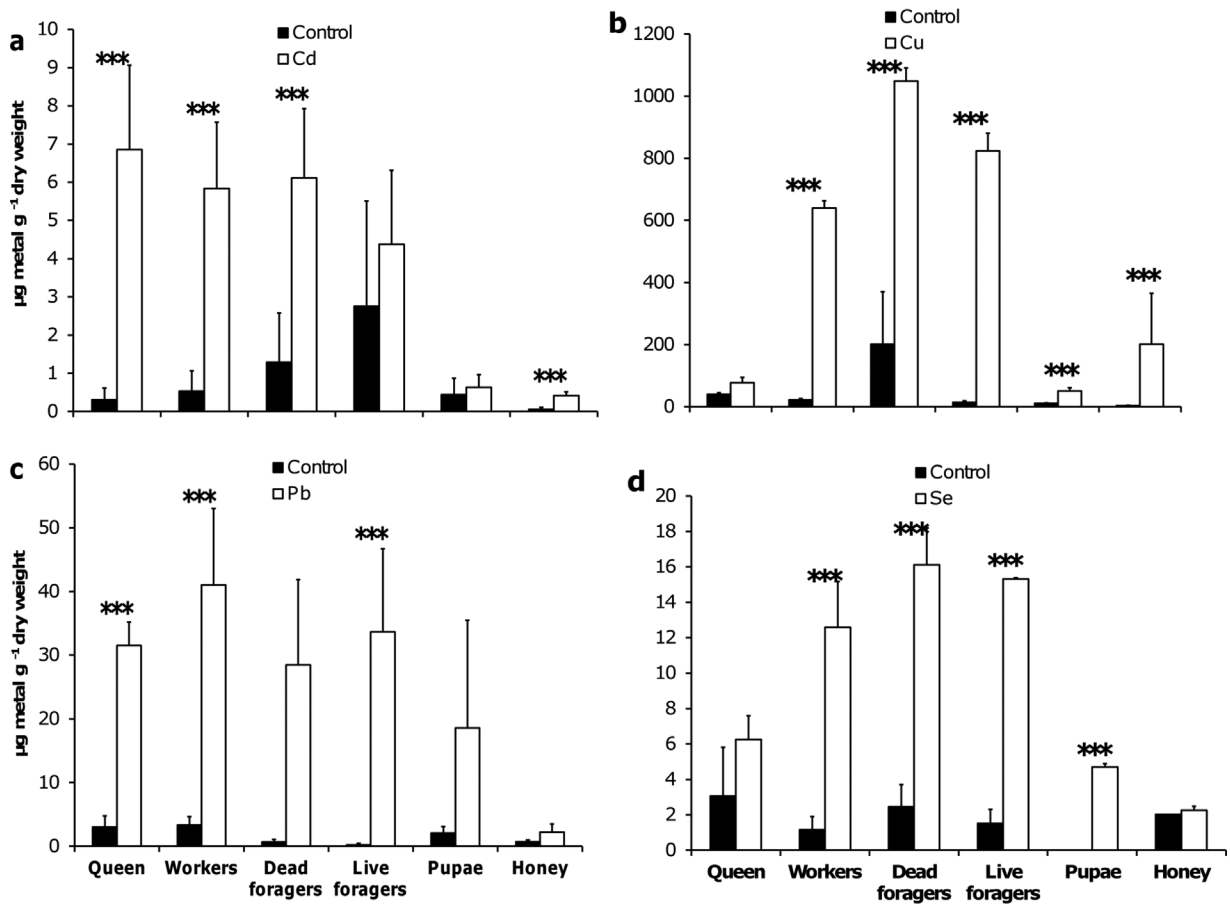

Figure 2. Elemental metal accumulation in colony members treated with control and (**a**) Cd, (**b**) Cu, (**c**) Pb, and (**d**) Se. Bars (mean ± standard error) with *** are significantly different from control at the $p < 0.05$ level (Dunnett's test). Average insect tissue and honey weights were as follows: queens, 0.181 ± 0.012 g; workers, 0.181 ± 0.012 g; dead foragers, 0.153 ± 0.004 g; live foragers, 0.154 ± 0.003 g; pupae, 0.114 ± 0.007 g; honey, 0.327 ± 0.04 g.

foragers, pupae, and honey did not accumulate substantial amounts of Pb ($p > 0.08$; Figure 2c).

Colonies treated with Se significantly accumulated the metal in workers ($F_{1,5} = 13.40, p < 0.02$), dead foragers ($F_{1,4} = 32.01$, $p < 0.01$), live foragers ($F_{1,3} = 181.38$, $p < 0.001$), and pupae ($F_{1,2} = 836.05, p < 0.01$). Queens and honey did not accumulate significant quantities of Se ($p > 0.41$; Figure 2d).

Overall, workers accumulated all 4 metals. Queens did not accumulate much Cu or Se, but the brood absorbed significant quantities. Dead foragers found near the hive entrance tended to have higher metal accumulation than the other colony members (except for Pb).

DISCUSSION

The present study reveals the toxic effects of several metal contaminants on the honey bee colony as a whole. Honey bee colonies were restricted to feeding on sugar syrup and pollen patties spiked with Cd, Cu, Pb, and Se at concentrations similar to those measured in the honey and flowers collected from hives at contaminated sites. Although the brood surface area and colony weights were unaffected by treatments, honey and brood production were impacted. Colonies treated with Cd produced more honey surface area than controls. A possible explanation could be that with fewer live brood the honey would go unused compared with other treatments. The bees reduced down the Cd-spiked sugar syrup to honey, which increased the concentration of 0.24 mg Cd/kg in sugar syrup to 0.41 mg Cd/kg in stored honey, almost twice as high. The ingestion of contaminated honey could cause a malaise effect in workers, causing them to consume less of the honey subsequently [27,28]. Foragers fed Se experienced a malaise effect and consumed less sucrose for up to 5 d after dosing [23].

By the conclusion of the experiment, Se-treated colonies produced no brood. In previous work, high mortality occurred when honey bees were exposed to Se at levels as low as 0.72 mg/L and development slowed at levels as low as 0.6 mg/L [23]. In addition, mortality occurred during the early instars, long before brood would be capped. At the end of the experiment, frames contained no capped brood. This could be explained if the capped brood had all emerged or if, following chronic exposure to Se, all the brood had died early on. Examination of the comb from repeated photographic records indicated that early brood mortality was the likely cause. Colonies treated with Se also had a significant reduction in total worker weight, and this could be explained by far fewer larvae surviving to adulthood or dying soon after emergence. Selenium had reduced the overall adult population of the hive, which can cause dire consequences for resource gathering, care for brood, and other critical tasks provided by the workers. If the adult population is reduced because of forager losses, this can destabilize hive demography and lead to colony failure [29].

With an average development time of 24 d from egg to adult emergence [30], bees would have been given enough time to create a new generation at least once in the experiment's 60-d duration. Cadmium and Cu treatments had more capped brood

and dead pupae compared with control hives at the same time point. However, total worker weights at the end of the experiment were similar for Cd, Cu, and control. This may have occurred if the Cd and Cu colonies produced more brood than controls to compensate for losses. Although there were similar worker weights when compared with control, the Cd and Cu results may translate to reduced overall worker populations over time periods longer than 60 d. With prolonged chronic exposure, honey bee hives in metal-contaminated environments may accumulate higher concentrations, experience increased brood mortality, and finally produce fewer colony members overall.

Although foragers tended to accumulate more metal in the present study compared with foragers collected at contaminated sites (Table 1), the present study represents a worst-case scenario of a honey bee hive fed exclusively a contaminated food source. Honey bees have an extensive foraging range of up to 7 km^2 [31] and may be more likely to encounter uncontaminated food resources to dilute any toxicants, although foraging bees do not prefer food with reduced concentrations of Se [32]. However, avoiding uncontaminated food sources may be more difficult when a contaminant occurs over large areas, such as Se in the western United States [7,13]. Also, metal contaminants can be more persistent than pesticides in the environment and, therefore, chronically expose the honey bee hive over time. As foragers continue to sample the environment, metals are deposited from the atmosphere onto the hairs of bee bodies or on the entire hive itself. The bees can also collect contaminated resources from metal-accumulating plants and even water sources [33]. Bromenshenk et al. [18] found similar results using nucleus colonies placed near industrialized regions. Colony performance was reduced when they were placed close to the point source of metal pollution. Sites closest to the contamination source produced lower honey stores, fewer adult bees, less brood, and less wax over time. Foragers in these polluted areas contained up to 5.5 mg Cd/kg [18], which is approximately what was found in the present study. These sites were contaminated with both Cd and arsenic; thus, the toxic effects may not have been from Cd alone. We observed reductions in honey stores, brood survival, and worker population weights when exposing the colonies to metals individually. Often, these contaminants co-occur in the environment and may cause more drastic, synergistic effects on whole-colony health. Mosquitoes exposed to Se and mercury, 2 contaminants that can co-occur in polluted waterways, suffered higher mortality in combination than from each metal alone [33]. Honey bee hives in contaminated areas often contain more than 1 metal contaminant [34–36], and in some cases, Cd, Cu, Pb, and Se were all found in the same hives [36]. Industrial and mining areas often disperse more than 1 metal into the environment [37], thus simultaneously exposing honey bee hives in the area.

Pollen patty consumption in Cu-treated and Se-treated hives was reduced, and this may have been the result of fewer live brood and workers at the conclusion of the experiment. Pollen patty resources tend to be consumed right away rather than stored [38,39], and the continuous dosing of brood and nurse bees may have slowed development, caused high mortality in brood, and prevented new adults from emerging in Se treatments.

All queens remained alive at the conclusion of the experiment. Queen egg laying was probably not affected by Cd, Cu, or Pb treatments, because there was no difference in total worker weights. Colonies treated with Cd or Cu had substantial amounts of capped brood but lower brood survival compared to control. Interestingly, queens did not accumulate significantly more Se or Cu compared to controls. Some insects biotransfer the Se to the eggs [40] as a means of detoxification. The queens may have lowered their own body burden by maternally transferring Se into the eggs, reducing their viability and eventually the worker numbers. There is currently no information about maternal transfer of Cd, Cu, or Pb toxicants to eggs in insects. However, queens exposed to Cu also did not accumulate substantial quantities of the metal, suggesting that egg laying was a means of excreting excess Cu.

The only metals that significantly accumulated in the pupae were Cu and Se. Insects are able to eliminate some amount of the metal in their myconium prior to pupation or right after emergence [41], but in the present study these metals remained inside the older brood. Contrary to Cu accumulation in gypsy moth [42] and Cd accumulation in flesh fly [41] and midge [43], honey bee adults (workers and foragers) accumulated higher concentrations of all 4 metals compared with the pupae. Workers and foragers were not starved prior to freezing for metal analysis and may have had a crop full of contaminated sugar syrup. Mechanisms for detoxification such as metallothioneins [44,45] and metal-sequestering granules that are frequently seen in other invertebrates [46,47] are used as biomarkers for metal exposure [48]. However, honey bees do not possess as many detoxification genes as other insects [49] and may utilize a first line of defense that incorporates caste structure, behavior, and dilution of toxicants in these social insects [50].

The present study is the first controlled experiment to explicitly test the effects of metals on whole honey bee colony health at the levels found in contaminated areas. Our exposure time of approximately 6 wk continuously feeding a contaminated food source is conservative in that honey bee hives exist in the polluted environment for several seasons, and metals do not break down or have reduced activity over time like some pesticides. During the short duration of the present study, hives experienced reduced total worker weights when treated with Se and reduced pupal survival when treated with Cd or Cu. Over an extended period of time, overall population numbers could drop further with continued exposure to these metal contaminants. Certain metals may be found not only in the floral resources gathered by foragers but also in the water and soils surrounding the colony. Temporal and spatial factors can add variation when sampling contaminants from honey bees in the environment [51,52]. In the present study, by eliminating these environmental factors, we were able to pinpoint the direct effect of each metal on honey bee colony health. If migratory honey bee hives remain in a metal-contaminated area for an extended period of time, they may suffer high brood mortality, leading to less adult emergence. Beekeepers could move hives out of contaminated areas to help the bees recover from being dosed with metals from the environment. In addition, metals may contribute to the overall toxic burden honey bees face when attempting to pollinate pesticide-treated agricultural fields. If honey bee hives are temporarily relieved from the burden of metal exposure, they may be able to recover, as has been seen for interrupted bumblebee exposure to imidacloprid [53]. Metals and pesticides could have an additive or even a synergistic effect on reducing colony performance. Since the 1930s, honey bees have mainly been used to detect environmental contamination [54] because of their ability to gather contaminated materials from air, plants, and water and return it to a central location (the honey bee hive). The present study reveals the whole-colony impact of the

environmental contaminants Cd, Cu, Pb, and Se using honey bees that were previously researched only for their role as bioindicators.

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Data availability—Data can be requested by contacting the corresponding author at kristen.hladun@ucr.edu

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