Title

Effects of trace metals on *Bombus terrestris* (Hymenoptera: Apidae) microcolony and excretion during larval development

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TS: Methodology, Investigation, Writing - Review & Editing. **TM**: Methodology, Investigation, Writing - Review & Editing. **LM**: Methodology, Investigation, Resources, Writing - Review & Editing. **HD**: Methodology, Investigation, Resources, Writing - Review & Editing. **DM**: Resources, Writing - Review & Editing. **KT**: Resources, Funding Acquisition, Writing - Review & Editing. **AG**: Conceptualization, Methodology, Validation, Formal Analysis, Investigation,

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Abstract

Trace metal pollution is an increasing threat to wildlife, driven by the growing demand for these elements in technologies. Because metals contaminate floral resources, they represent a major but poorly understood risk for bee communities, including social species such as bumble bees (Hymenoptera: Apidae). In particular, the sublethal consequences of metal exposure for bumble bee colony development remain scarcely documented, and the associated metal excretion process occurring at the larva-pupa interface in these holometabolous insects is still unclear. Here, we exposed queenless microcolonies of the buff-tailed bumble bee *Bombus terrestris* (L.) to field-realistic concentrations of copper (692 μM) or cadmium (7.38 μM), and assessed their effects on sucrose syrup consumption, worker survival, egg-laying event and brood development. We also quantified metal concentrations in larval and pre-pupal tissues to investigate potential metal excretion during development. Copper, but not cadmium, had detrimental effects on microcolony performance, as shown by reduced worker survival, delayed egg laying and impaired brood development. Workers exposed to copper also reduced their consumption of contaminated sucrose syrup, suggesting a possible behavioural avoidance to mitigate toxicity. In addition, metals were found at higher concentrations in larval than in pre-pupal tissues, indicating that metal excretion likely occurs at the larval-pupal interface. Overall, our study strengthens current knowledge on the effects of trace metals in social bees and provides evidence for a potentially important excretion process occurring during larval development.

Keywords

Bumblebee; Contamination; Detoxification, Heavy metals; Metamorphosis

Introduction

The Earth system has now crossed six of the nine "planetary boundaries" (Richardson *et al.* 2023), including chemical pollution, which poses major risks to human health and wildlife (Michelangeli *et al.* 2022; Naidu *et al.* 2021). Among chemical contaminants, trace metals (often misleadingly called "heavy metals") are widespread micropollutants whose concentrations have risen well above natural background levels due to intensive human activities such as mine tailing, agrochemical spread, road traffic and the production of electronic devices (Bertram *et al.* 2022; Vareda *et al.* 2019). Globally, an estimated 17% of cropland contains toxic levels of trace metals, threatening the health of over one billion people (Hou *et al.* 2025). Metals present in soils and water can accumulate in zoophilous plants, including their floral resources, creating a direct route of exposure for pollinators (Scott & Gardiner 2025). Bees, one of the most important pollinator groups (Porto *et al.* 2020), are therefore at high risk, as reflected by the accumulation of trace metals in the nests of sentinel species (Benner *et al.* 2025; Goretti *et al.* 2020).

Due to their high affinity for electron-dense sites such as DNA and cysteine residues in proteins, as well as their strong potential to disrupt redox balance (Valko *et al.* 2005), trace metals can cause severe sublethal damage in bees (e.g., Bernardes *et al.* 2021), with cascading effects from the individual (e.g., Li *et al.* 2022) to the community level (e.g., Shi *et al.* 2023). Despite increasing recognition of their detrimental impacts on bees (Gekière *et al.* 2023b), the effects of these pollutants on colony development in social bees remain poorly understood. Social bees are primarily corbiculate bees within the Apidae family, which includes honey bees, bumble bees and stingless bees (Michez *et al.* 2019). These species exhibit division of labour and alloparental brood care, with a reproductive queen laying eggs and non-reproductive workers foraging and nursing the brood, ultimately leading to the production of males and virgin queens (Cardinal & Danforth 2011). In bumble bees, experimental studies have shown

that trace metal exposure can disrupt colony development, with impacts on both adults and larvae (Scott *et al.* 2022; Sivakoff *et al.* 2020; Wu *et al.* 2025).

As other terrestrial insects, bees may mitigate trace metal toxicity through four main mechanisms, namely avoidance, barrier, detoxification/excretion and alleviation (Gekière 2025), yet detoxification and excretion process remaining poorly understood. In adult bees, trace metals are likely scavenged by metal-binding proteins and subsequently excreted via the Malpighian tubules (Borsuk *et al.* 2021). Alternatively, metals may be sequestered in gut epithelial cells and later eliminated through desquamation and defecation, thereby preventing their entry into the hemolymph (Rost-Roszkowska *et al.* 2008). In contrast, bee larvae defecate only at the onset of pupation (i.e., pre-pupae), suggesting that metals may accumulate throughout larval development and be expelled in substantial quantities only before pupation. Another potential detoxification mechanism during this developmental transition is the incorporation of metals into the cuticle, followed by their removal through moulting or metamorphosis, a process previously documented in several beetle species (Lindqvist *et al.* 1995; Przybyłowicz *et al.* 2003). However, such metal elimination at the larva-pupa interface has not yet been investigated in bees. In bumble bees, queenless microcolonies offer a powerful experimental system to investigate this hypothesis, as their reduced brood size and simplified organisation allow larvae and pre-pupae to be more easily located and sampled at precise timing, allowing direct measurement and comparison of metal bioaccumulation across developmental stages.

However, no study to date has investigated the effects of trace metals on bumble bee development using a microcolony design. Queenless bumble bee microcolonies consist exclusively of sister workers, among which one or more individuals activate their ovaries and lay unfertilised male-destined eggs (i.e., haplodiploidy) in the absence of queen pheromones. These systems serve as convenient surrogates for queenright colonies, offering fully controlled

99 experimental conditions, high standardisation and the possibility of large replication.
100 Importantly, the simplified social structure of microcolonies allows for straightforward
101 monitoring of key developmental parameters, such as food consumption, egg laying, oophagy,
102 brood emergence, and worker mortality, whereas these metrics are logistically challenging to
103 measure in fully developed colonies with a queen (Klinger *et al.* 2019; Tasei & Aupinel 2008).
104 Owing to these advantages, microcolonies have been widely employed to assess the effects of
105 diverse stressors, including pesticides (Krueger *et al.* 2021), parasites (Gekière *et al.* 2023a)
106 and plant specialised metabolites (Tourbez *et al.* 2023), yet their application to test the effects
107 of trace metals remains unexplored.

108 In this study, we exposed queenless microcolonies of the buff-tailed bumble bee *Bombus*
109 *terrestris* (L.) (Hymenoptera: Apidae) to field-realistic concentrations of copper and cadmium,
110 two trace metals that are both widespread and environmentally relevant (Comber *et al.* 2023;
111 Nagajyoti *et al.* 2010), for a period of 28 days. To assess the effects of trace metal exposure on
112 microcolony development, we monitored food consumption, egg-laying events, oophagy, male
113 emergence and worker survival throughout the experiment. At the end of the exposure period,
114 microcolonies were dissected to evaluate brood development. In parallel, larvae and pre-pupae
115 were sampled from each microcolony, and metal concentrations in their bodies were quantified
116 to compare metal accumulations across developmental stages. We hypothesised that trace metal
117 exposure would negatively impact microcolony development and that larvae would eliminate
118 accumulated metals before pupation, resulting in lower metal concentrations in pre-pupae
119 compared to larvae.

120 **Material and Methods**

121 ***Bumblebee breeding and exposure***

122 Five standard colonies of *Bombus terrestris* (~50 workers per colony upon receival) were
123 obtained from the commercial supplier Biobest (Westerlo, Belgium) and fed *ad libitum* with

Biogluc® sugar solution. Each colony was used to implement six microcolonies that consisted of five sister workers placed in plastic boxes (10 × 16 × 16 cm). These microcolonies were distributed among three treatments (i.e., 10 microcolonies per treatment) by ensuring homogeneity of origins (i.e., all colonies were equally represented in each treatment). Microcolonies were reared in a dark room under constant conditions (27 ± 1 °C and 60 ± 10% relative humidity) for 28 days.

Throughout the experiment, microcolonies were provided *ad libitum* with the same multifloral pollen (5:2 pollen:water w/w) and their respective treatment in 50% sugar solution (1:1 sucrose:water w/w; see below). Pollen and sugar solutions were weighed and replenished every four days to quantify food consumption over time. To correct for evaporation, an additional box maintained under identical conditions but without bees served as an evaporation control for pollen and sugar solutions. Corrected food consumption was calculated by subtracting evaporation losses from observed consumption and subsequently standardised to the total worker mass within each microcolony (g food/g bee).

Reproductive and survival parameters were also recorded, including the date of first egg laying, the date of first male emergence, and worker mortality. Dead individuals were weighed and replaced with colour-tagged workers from the same colony to maintain a constant number of workers across microcolonies and control for the indirect effects of worker mortality on brood development. The replacement of dead workers was performed throughout the 28-day experimental period. Oophagy was noted when no larvae were observed 10 days after egg laying, indicating egg consumption by workers. To assess metal accumulation during development, three larvae were sampled 10 days after egg laying, while the first three pre-pupae were collected upon reaching the pre-pupal stage, per microcolony and stored at –20 °C until further analyses. Because larval development was reduced in the copper treatments, only larvae and pre-pupae from the control and cadmium treatments could be sampled.

149 At the end of the experiment, microcolonies were frozen at –20 °C and dissected. For
150 each microcolony, we recorded the number of eggs, larvae (including pre-pupae), pupae and
151 emerged males, as well as the total biomass of larvae (including pre-pupae), pupae and emerged
152 males separately.

153 ***Treatments***

154 Microcolonies were provided *ad libitum* with 50% sugar solution (1:1 sucrose:water w/w) with
155 either (i) no metal, (ii) 93 $mg.L^{-1}$ of copper chloride ($CuCl_2$; Sigma-Aldrich, CAS 7447-39−4)
156 equivalent to 44 $mg.L^{-1}$ of copper (692 µM), or (iii) 1.35 $mg.L^{-1}$ of cadmium chloride ($CdCl_2$;
157 Sigma-Aldrich, CAS 10108-64−2) equivalent to 0.83 $mg.L^{-1}$ of cadmium (7.38 µM). Salt
158 powders were weighed (± 0.1 mg) and directly dissolved in 50% sugar solution. Chloride salts
159 were used as soluble metal sources, and the observed biological effects are expected to primarily
160 reflect exposure to copper and cadmium cations rather than to chloride anions (Bounias *et al.*
161 1996; Monchanin *et al.* 2022). Metal concentrations were measured at the Mineral and Organic
162 Chemical Analysis platform (MOCA; Louvain-la-Neuve, Belgium) using Inductively Coupled
163 Plasma - Optical Emission Spectrometer (Agilent 5800 VDV ICP-OES). These concentrations
164 align with concentrations of copper and cadmium found in the beebread of buff-tailed bumble
165 bees or in floral resources, thereby mimicking environmentally realistic exposure levels
166 (Sivakoff *et al.* 2020; Xun *et al.* 2018).

167 ***Metal analysis***

168 Bioaccumulation of cadmium in larvae and pupae was measured following a protocol adapted
169 from Scott *et al.* (2024). Thawed samples were weighed (± 0.1 mg), rinsed with ultrapure water
170 to remove surface metals and then dried in an oven at 60 °C for 72 h. Dried specimens were
171 ground to a fine powder using a pestle to ensure homogenisation, after which the homogenate
172 was further dried at 60 °C for 48 h and weighed (± 0.1 mg) to determine the fresh-to-dry mass
173 ratio (larvae: 3.72 ± 0.42 with n = 56; pupae: 3.34 ± 0.45 with n = 47; mean ± SD).

Approximately 60 mg of ground dry tissue was weighed (± 0.1 mg) into a 50 mL glass beaker. Sample digestion was performed using 65% Suprapur® nitric acid (HNO_3; Merck, CAS 7697-37-2) and 30% Suprapur® hydrogen peroxide (H_2O_2; Sigma-Aldrich, CAS 7722-84-1). The digestion procedure consisted of the following steps: (i) addition of 2 mL HNO_3 and 2 mL H_2O_2 (dropwise) on a hot plate at 70 °C for 1 h, (ii) addition of 2 mL H_2O_2 (dropwise) at 70 °C for 30 min, and (iii) addition of 2 mL H_2O_2 (dropwise) at 70 °C for 1 h. Following complete digestion, samples were evaporated on a hot plate at 120 °C until dryness. The residues were then resuspended in 10 mL of 2% (v/v) Suprapur® nitric acid (HNO_3; Merck, CAS 7697-37-2) and stored at room temperature for a maximum of one week until analysis. Concentrations were measured at the Mineral and Organic Chemical Analysis platform (MOCA; Louvain-la-Neuve, Belgium) using Inductively Coupled Plasma - Mass Spectrometry (Thermo Scientific iCAP Q ICP-MS). Three larval and three pre-pupal samples per microcolony were analysed individually (i.e., analytical triplicates) and we used the mean concentration of these three samples for statistical analyses. Concentrations are reported for dry tissues.

Statistics

Count variables (egg count, larval count, pupal count, male count and total brood count), biomass variables (i.e., larval mass, pupal mass and total brood mass), food collection (total syrup and pollen collection), oophagy and metal concentrations in tissues were analysed using (generalised) linear mixed-effects models ((G)LMERs). For all variables except metal concentrations in tissues, treatment was specified as a fixed effect and colony of origin was included as a random intercept. For metal concentrations in tissues, treatment, developmental stage and their interactions were specified as fixed effects, while microcolony nested within colony of origin was included as a random intercept. Biomass, food collection and metal concentration data were fitted with LMER (i.e., Gaussian error distribution and identity link) using the lmer() function from the *lme4* package (Bates *et al.* 2021). Count data were analysed

with GLMER with a negative binomial distribution and a log link, whereas oophagy was analysed with a binomial distribution and a logit link. All GLMERs were implemented with the glmmTMB() function from the *glmmTMB* package (Brooks *et al.* 2017).

Worker survival and timing of first egg-laying were analysed with Cox proportional hazards models, including treatment as a fixed effect and colony of origin as a random intercept, using the coxph() function from the *survival* package (Therneau 2021).

When significant treatment effects were detected ($p < 0.05$), pairwise *post-hoc* comparisons were performed with the emmeans() function from the *emmeans* package (Lenth 2022). Model diagnostics were conducted using the simulateResiduals() function from the *DHARMa* package (Hartig 2021) for (G)LMERs, and the proportional hazards assumption was assessed with the cox.zph() function from the *survival* package (Therneau 2021) for Cox models.

All figures were produced with the ggplot() function from the *ggplot2* package (Wickham *et al.* 2020) and the ggsurvplot() function from the *survminer* package (Kassambara *et al.* 2021). Statistical analyses were performed in R version 4.4.0 (R Core Team 2024).

Results

We detected a significant effect of treatment on multiple aspects of microcolony development (**Table 1**). Microcolonies exposed to copper exhibited consistently impaired performance compared with control and cadmium-exposed microcolonies, whereas no significant differences were observed between the control and cadmium-exposed microcolonies. Copper exposure significantly reduced brood production, as reflected in egg ($\chi^2 = 15.50$, $df = 2$, $p < 0.001$), larval ($\chi^2 = 19.20$, $df = 2$, $p < 0.001$), pupal ($\chi^2 = 8.98$, $df = 2$, $p = 0.011$) and total brood counts ($\chi^2 = 22.80$, $df = 2$, $p < 0.001$; **Figure 1A**). Similarly, brood biomass was affected, with copper-exposed microcolonies showing lower larval mass ($\chi^2 = 34.45$, $df = 2$, $p < 0.001$), pupal mass ($\chi^2 = 15.46$, $df = 2$, $p < 0.001$), and total brood mass ($\chi^2 = 38.17$, $df = 2$, $p < 0.001$; **Figure**

1B). Copper also delayed the onset of egg laying ($\chi^2 = 9.09$, df = 2, $p = 0.011$; **Figure 2A**), increased original worker mortality ($\chi^2 = 113.59$, df = 2, $p < 0.001$; **Figure 2B**), and reduced resource collection, both for syrup ($\chi^2 = 19.14$, df = 2, $p < 0.001$; **Figure 3A**) and pollen ($\chi^2 = 22.29$, df = 2, $p < 0.001$; **Figure 3B**). In contrast, neither the number of emerged males ($\chi^2 = 4.34$, df = 2, $p = 0.114$) nor the occurrence of oophagy ($\chi^2 = 4.71$, df = 2, $p = 0.094$) differed significantly among treatments.

Because larval development was reduced in the copper treatments, only larvae and pre-pupae from the control and cadmium treatments could be analysed for metal concentrations in tissues. Analysis of cadmium concentrations in bee tissues revealed significant effects of treatment ($\chi^2 = 21.00$, df = 1, $p < 0.001$) and developmental stage ($\chi^2 = 16.80$, df = 1, $p < 0.001$), as well as a significant interaction between treatment and developmental stage ($\chi^2 = 10.3$, df = 1, $p = 0.001$), indicating treatment-dependent differences in cadmium accumulation between larvae and pre-pupae (**Figure 4**). In control microcolonies, cadmium concentrations did not differ between larval tissues (mean ± SD: 0.59 ± 0.34 µg / g dry tissue) and pre-pupal tissues (mean ± SD: 0.46 ± 0.24 µg mg / g dry tissue). In contrast, in cadmium-exposed microcolonies, larval tissues harboured significantly higher cadmium concentrations (mean ± SD: 1.95 ± 0.67 µg / g dry tissue) than pre-pupal tissues (mean ± SD: 0.68 ± 0.56 µg / g dry tissue).

Discussion

Using microcolonies of the buff-tailed bumblebee *Bombus terrestris* exposed to field-realistic concentrations of trace metals for 28 days, our study highlights significant detrimental effects of copper on worker survival, egg laying, brood development and food consumption, whereas microcolonies exposed to cadmium did not differ from control microcolonies. These results suggest that field-realistic concentrations of trace metals can exert detrimental effects on bumble bee colonies, depending on the metal considered. In addition, by analysing trace metal concentrations in larval and pre-pupal tissues, we observed a significant decrease in cadmium

concentrations during the transition from larvae to pre-pupae, suggesting the occurrence of excretion processes during this transitional stage.

Field-realistic exposure to copper (692 µM) caused detrimental effects on worker survival, egg laying and brood development in queenless microcolonies of *B. terrestris*. These findings are consistent with previous work showing that copper-contaminated pollen and sucrose syrup negatively affected egg laying and brood development in queenright *B. terrestris* colonies, even at lower concentrations than those used in our study (157-471 µM in syrup; Wu *et al.* 2025). While that study did not report significant effects on queen mortality, the comparison suggests that our queenless microcolony design effectively captures the sublethal and colony-level effects of copper that are relevant in full queenright colonies. By contrast, we observed no detrimental effects of a field-realistic concentration of cadmium (7.38 µM) on worker survival, egg laying or brood development in queenless microcolonies of *B. terrestris*. These findings are consistent with a previous semi-field study exposing queenright colonies of *B. impatiens* to a similar cadmium concentration (1.51 µM), which reported no significant impact on overall brood development, such as brood count after 30 days, although a higher larval mortality was observed throughout the experiment (Scott *et al.* 2022). It is important to note, however, that a microcolony setup prevents the assessment of impacts on reproductive individuals, namely the production of new queens and males (Van Oystaeyen *et al.* 2021), which is critical for understanding the mechanisms driving population declines in bumble bees (Gekière *et al.* 2025a). Cadmium may therefore have detrimental consequences on the production of those reproductive individuals, and future studies relying on queenright setups should explore such potential effects, since it was not recorded in previous studies relying on queenright colonies (Scott *et al.* 2022; Wu *et al.* 2025).

Regarding syrup consumption, we found that *B. terrestris* workers reduced their intake of copper-contaminated sucrose solution, whereas no such reduction was observed with

cadmium-contaminated sucrose solution. As workers are unlikely to detect field-realistic concentrations of copper upon initial contact with the solution (Gekière *et al.* 2024), this observed pattern suggests that consumption was mediated by post-ingestive feedback rather than pre-ingestive cues. Two non-mutually exclusive mechanisms may explain this reduction. On the one hand, copper exposure may induce physiological or histological damages that mechanically constrain food intake (i.e., passive mechanism; Bernardes *et al.* 2021). On the other hand, copper may disrupt neural pathways involved in appetitive motivation, leading to an active reduction in feeding behaviour (i.e., active mechanism; Huang *et al.* 2022; Paoli *et al.* 2023). Given that workers can avoid metal-contaminated resources when provided with a choice by relying on spatial learning and post-ingestive reinforcement processes (Gekière *et al.* 2025b; Wright *et al.* 2010), it is likely that, in the absence of alternative uncontaminated options, they actively reduce consumption due to reinforcement-associated reduced appetitive motivation. Future studies investigating the regulation of neuromodulators such as dopamine and serotonin in bumble bees exposed to trace metals would help clarify the role of such active avoidance mechanisms (Wright *et al.* 2010).

Finally, in microcolonies exposed to cadmium-contaminated sucrose solution, we observed a substantial reduction in cadmium concentration in tissues between the larval and pre-pupal stages. This pattern is consistent with a previous study showing that cadmium bioaccumulation decreased from larvae to pupae in *B. impatiens* (Scott *et al.* 2024). Although the cadmium accumulation reported in that study was lower than the one measured here, this difference is likely explained by the lower cadmium concentration provided in the sucrose syrup (1.16 µM *versus* 7.38 µM in our study). Although Scott *et al.* (2024) focussed on pupae while we analysed pre-pupae, both studies converge on the same conclusion, namely that bumble bee larvae appear able to eliminate a substantial fraction of accumulated cadmium at the transition between the larval and pupal stages, suggesting a potentially important line of defence against

trace metal exposure (Gekière 2025). Two non-mutually exclusive mechanisms may explain this reduction in concentration in tissues: (i) the elimination of metals through gut flushing prior to pupation (i.e., meconium deposition; Jiang *et al.* 2018; Liu *et al.* 2025; Wu *et al.* 2020), or (ii) the elimination of metals during larval-pupal ecdysis (i.e., shedding of the larval cuticle), as reported in other insects such as leaf beetles and black soldier flies (Diener *et al.* 2015; Jiang *et al.* 2018; Przybyłowicz *et al.* 2003). In our study, because we analysed pre-pupae, which have not yet undergone larval-pupal ecdysis (Elias-Neto *et al.* 2009; Tian & Hines 2018), our results indicate that cadmium elimination had occurred through gut flushing at the larval-pre-pupal transition. Although metal concentrations have been quantified in the faeces and exuviae of other insect species (Diener *et al.* 2015; Urbini *et al.* 2006), future studies should directly measure trace metal concentrations in the meconium and exuviae of bumble bee larvae to better characterise these detoxification pathways.

Our study has three major limitations that should be addressed in future research. First, although we observed contrasting effects between copper- and cadmium-treated microcolonies, our design did not allow to determine whether these differences are metal- or concentration-dependent, as both factors are confounded. Indeed, while both treatments reflected field-realistic exposure levels, the copper concentration was more than 90 times higher than that of cadmium. Our results should therefore not be interpreted as evidence that copper has more detrimental sublethal effects than cadmium in bumble bees. Rather, comparative assessments of metal-specific toxicity will require experiments exposing bumble bees to equivalent concentrations of different metals. Second, our experiment was conducted under a no-choice design, representing a worst-case exposure scenario. Under natural conditions, bumble bee workers may encounter a range of resources differing in contamination levels, from heavily contaminated to uncontaminated floral sources (Scott & Gardiner 2025), and may adjust their foraging behaviour accordingly (Meindl & Ashman 2013; Xun *et al.* 2018). As such, our results

may overestimate actual exposure and associated effects when compared to what occurs in the field. Third, although we showed that bumble bee larvae likely eliminate cadmium prior to pupation through meconium deposition, our data do not reveal the underlying physiological mechanisms. In particular, it remains unclear whether metals are actively transported from internal tissues back into the gut lumen for excretion, as described for copper transport (DmATP7) in the midgut of *Drosophila melanogaster* (Burke *et al.* 2008), or whether the excreted metals simply correspond to metal fractions that remained within the gut and never crossed the gut barrier into body tissues. The recent availability of high-quality bumble bee genomes, together with targeted transcriptomic approaches, offers promising opportunities to identify and characterise the potential transporters involved in these processes (Crowley *et al.* 2023; Toth *et al.* 2024).

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Competing interests

The authors have no conflict of interest to declare.

Data availability

The dataset is publicly available on Zenodo - https://doi.org/10.5281/zenodo.20391517.

Figure 1. Effects of trace metals on brood development in *Bombus terrestris* microcolonies measured as total brood count (**A**) and total brood mass (**B**). Mean and standard error of the mean are plotted in black. Treatment not sharing the same letter show significant differences (GLMER for total brood count and LMER for total brood mass).

Figure 2. Kaplan-Meier curves showing the effects of trace metals on first egg laying (**A**) and worker survival (**B**) in *Bombus terrestris* microcolonies. Treatment not sharing the same letter show significant differences (Cox models for both panels).

Figure 3. Effects of trace metals on food consumption in *Bombus terrestris* microcolonies measured as standardised syrup consumption (**A**) and standardised pollen consumption (**B**). Mean and standard error of the mean are plotted in black. Treatment not sharing the same letter show significant differences (LMER for both panels).

Figure 4. Cadmium concentrations in the dry tissues of larvae and pre-pupae from control and cadmium-exposed *Bombus terrestris* microcolonies. Mean and standard error of the mean are plotted in black. The asterisk indicates a significant difference, whereas “n.s.” denotes a non-significant difference.

Table 1. Statistical results for *Bombus terrestris* microcolony development. Omnibus test with significant p-values indicate significant differences among treatments (< 0.05 in bold). For variables with a significant omnibus test, pairwise comparisons are reported. Significant p-values (< 0.05) are also shown in bold.

Response variable	χ^2	**Degree of freedom**	**Omnibus p-value**	**Ctrl – Cd contrast (p-value)**	**Ctrl - Cu contrast (p-value)**	**Cd - Cu contrast (p-value)**
Egg count	15.50	2	**<0.001**	0.22 (0.483)	1.92 **(<0.001)**	1.70 **(<0.001)**
Larval count	19.20	2	**<0.001**	-0.12 (0.689)	2.57 **(<0.001)**	2.69 **(<0.001)**
Pupal count	8.98	2	**0.011**	0.23 (0.553)	3.13 **(0.008)**	2.89 (**0.009)**
Male count	4.34	2	0.114	/	/	/
Total brood count	22.80	2	**<0.001**	0.02 (0.928)	2.22 **(<0.001)**	2.19 **(<0.001)**
Larval mass	34.45	2	**<0.001**	0.35 (0.446)	2.44 **(<0.001)**	2.09 **(<0.001)**
Pupal mass	15.46	2	**<0.001**	0.79 (0.213)	2.38 **(0.002)**	1.59 **(0.025)**
Total brood mass	38.17	2	**<0.001**	0.93 (0.271)	4.78 **(<0.001)**	3.86 **(<0.001)**
First egg laying	9.09	2	**0.011**	0.31 (0.509)	1.50 **(0.022)**	1.19 **(0.029)**
Oophagy	4.71	2	0.094	/	/	/
Survival	113.59	2	**<0.001**	1.17 (0.151)	-2.90 **(<0.001)**	-4.07 **(<0.001)**
Total syrup collection	19.14	2	**<0.001**	-1.23 (0.757)	14.27 **(0.002)**	15.51 **(0.002)**
Total pollen collection	22.29	2	**<0.001**	0.41 (0.686)	4.31 **(<0.001)**	3.90 **(0.001)**

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