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Scientific Note

Lead bioaccumulation in herbivorous insects and parasitoids reared on plants grown in lead-contaminated soil under field conditions

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Abstract. We investigated the lead (Pb) bioaccumulation in herbivorous insects and parasitoids on kale plants (*Brassica oleracea* var. acephala) cultivated in soils experimentally contaminated. We cultivated kale in soil with lead nitrate concentrations of 0 (control), 144, 360, and 600 mg/Kg of soil, representing permissible levels for Brazilian soils. The plants were kept in an open greenhouse to allow the natural colonization by insects under field conditions. We collected insects through direct removal or trap bags. Dried samples of leaves, herbivorous sap-sucking and chewing insects, and their respective parasitoids were analyzed utilizing ICP-OES to determine Pb concentrations. Pb was transferred in this system, with insects showing higher Pb content than leaves, and the highest values being found in parasitoids, which exhibited the highest levels ever recorded, even though our foliar Pb levels were lower than those in laboratory assays conducted up to then. These results indicate Pb biomagnification. We discuss Pb bioaccumulation effects on herbivores and parasitoids, comparing them with laboratory studies. We provide unprecedented insights into heavy metal bioaccumulation in field herbivorous insects and parasitoids.

Keywords: Aphids, Biomagnification, Heavy metals, Kale, Lepidopterans.

Heavy metals (HM) are highly toxic elements known for their propensity to accumulate in living tissues (Ali et al. 2019a). Some HM participate in vital biochemical activities for organisms, such as iron (Fe), copper (Cu), and zinc (Zn). However, when they reach concentrations above ideal levels, they become toxic. Other HM, such as lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr), do not play a role in the functioning of organisms and can be toxic even at low concentrations (Edelstein & Ben-Hur 2018). These metals naturally occur in soils and rocks at low concentrations. However, soil contamination with HM is often associated with mining and industrial activities, waste disposal, and indiscriminate use of agricultural inputs, like fertilizers and pesticides, among other activities (Chaffai & Koyama 2011; Wuana & Okieimen 2011).

HM from soil can be absorbed by plants and thus transferred throughout terrestrial food chains. Plants can transfer HM accumulated in their tissues to herbivorous insects, which, in turn, can transfer these elements to their natural enemies, such as predators and parasitoids (Kazimírová & Ortel 2000; Ye et al. 2009; Dar et al. 2017; Woźniak et al. 2017; Naikoo et al. 2021). In the bioaccumulation of HM, due to their cumulative potential, the concentration of these elements can be magnified in organisms along the food chains, characterizing biomagnification (Woźniak et al. 2017; Naikoo et al. 2021). On the other hand, some species can develop detoxification strategies, leading to a reduction in the concentration of these elements, characterizing biodilution or biominimization (Kazimírová & Ortel 2000; Ye et al. 2009; Naikoo et al. 2021).

The bioaccumulation of HM in herbivorous insects and parasitoids has been primarily studied through laboratory assays that often employ diets supplemented with HM as an initial food source (Ortel et al. 1993; Ortel 1995; Kazimirova et al. 1997; Kazimírová & Ortel 2000). Consequently, there is a gap in understanding how the bioaccumulation of HM occurs in these insects in natural systems and under field conditions. In light of this, the objective of the present study was to assess the Pb bioaccumulation in herbivorous insects and parasitoids reared on kale (*Brassica oleracea* var. acephala) cultivated in soils experimentally contaminated under field conditions.

We cultivated kale plants in soil contaminated with lead nitrate ($Pb(NO_3)_2$) at concentrations of 0 (control, referred to as T0), 144 (T1), 360 (T2), and 600 (T3) $mg.Kg^{-1}$ of soil, corresponding, respectively, to the prevention value, the agricultural area's limit value, and the residential area's Pb limit value, according to CONAMA (Brazilian National Environment Council) resolution 460/2013 (CONAMA 2013). We performed the contamination by diluting pre-weighed lead nitrate in 2 liters of clean water, used for plant irrigation, and applying it to the soil in the cultivation pots (Polypropylene, 12 liters). For the T0, only water was applied. To prepare the soil in each pot, we used the following mixture: 2 kg of expanded, used for drainage, and 8 kg of soil containing sand, animal-based organic fertilizer, and common limestone. We installed a hose connected to a PET bottle at the bottom of each pot, thereby closing the water circuit and preventing local contamination.

On January 9, 2019, we planted kale seedlings after three months of soil contamination to enhance Pb adherence. Ten plants per treatment, totaling 40, were grown individually in pots within an open greenhouse to allow natural insect colonization. The plants were randomly arranged with a 1.2 m^2 distance between them, and they were irrigated twice a week. The experiment took place at the Federal University of Lavras (UFLA) experimental farm in Ijaci, MG, Brazil.

We employed two methodologies for insect collection due to variations in insect colonization and feeding behaviors. We collected chewing herbivorous, specifically *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) and *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae), by inspecting each plant and gathering pupae in plastic tubes. These pupae were then brought to the laboratory for the emergence of adult herbivores or parasitoids [*Oomyzus sokolowskii* (Kurdjumov, 1912) (Hymenoptera: Eulophidae)]. We performed two collections, on March 28 and April 4, 2019. We used voile trap bags with a string at the base to collect sap-sucking herbivores, specifically *Brevicoryne brassicae* (Linnaeus, 1758) and *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), by bagging one already-infested kale leaf per plant, chosen at random, for 15 days in the field. Afterward, we took the bagged leaves to the laboratory to obtain the aphids and their emerging parasitoids [*Diaeretiella rapae* (McIntosh, 1855), *Lysiphlebus testaceipes* (Cresson, 1880) (Hymenoptera: Braconidae), *Aphelinus asychis* Walker, 1839 (Hymenoptera: Aphelinidae)]. We performed two collections, on May 13 and May 31, 2019.

After insect collections, we harvested the plants and transported them to the laboratory. Samples of dried and ground leaves and insects underwent Pb content analysis using an ICP-OES Optical Emission Spectrometer by the specialized company "SG Soluções Científicas" (São Carlos, SP, Brazil). Each plant's leaf sample weighed 1.0 g (dry weight), with 10 repetitions per treatment. Insect samples ranged from 0.1 to 0.35 g (dry weight). Aphid samples ranged from 6 to 10 per treatment. Due to their low mass, individual samples per plant were unattainable for lepidopterans, lepidopteran parasitoids, or aphid parasitoids, so we combined them into a single sample per treatment. Plant and aphid Pb data were analyzed using GLMs and Tukey's post-hoc test, following Gaussian distribution for plants and Gamma for aphids. Statistical analyses were performed in the R software (R Core Team 2020). Please refer to Supplementary Material for methodological details.

The average concentration of Pb in the leaf samples was: T0 = 0.017 (SD±0.054), T1 = 0.120 (±0.036), T2 = 0.181 (±0.125), and T3 = 0.388 (±0.150) mg of Pb.Kg^{-1}. Leaf samples from the most contaminated treatments showed Pb concentrations above the permitted limit of 0.3 mg.Kg^{-1} for kale intended for human consumption (ANVISA 2021; CODEX 2017). Data related to Pb concentration in plants were previously published by Morales-Silva et al. (2022).

In general, the average concentrations of Pb were higher in herbivores than in the plant (Fig. 1A). The average concentrations of Pb in aphids were: T0 = 0.711 (median 0.330, SD±0.706), T1 = 0.466 (0.466, ±0.136), T2 = 1.089 (1.069, ±0.755), and T3 = 0.599 (0.616, ±0.075) mg of Pb.Kg^{-1} (Fig. 1B; Supplementary material, Fig. S2). There were no statistically significant differences in Pb concentrations among treatments. Aphids showed higher Pb concentrations than kale samples, ranging from 0 to 2.45 mg.Kg^{-1} (besides the outlier of 10.71 mg.Kg^{-1}), while kale leaves ranged from 0 to 0.60 mg.Kg^{-1}. These results indicate that aphids are capable of bioaccumulating Pb from the kale, resulting in higher levels of accumulation than the plants (i.e., biomagnification). Studies have indicated that HM bioaccumulation in aphids varies with plant and aphid species and the specific metals involved. For instance, the study by Dar et al. (2017) found Cd and Zn biomagnified in *Lipaphis erysimi* (Kaltenbach, 1843) (Hemiptera: Aphididae) on mustard (*Brassica juncea* L.), but Pb decreased. In contrast, Woźniak et al. (2017) showed Pb biomagnification in *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) on pea (*Pisum sativum* L.). Our results indicate lower Pb concentrations in aphids than in the mentioned studies. Dar et al. (2017) found a maximum Pb concentration of 15.58 mg.Kg^{-1} in aphids that feed on mustards growing in soil amended with fly ash from a thermal power plant. Woźniak et al. (2017) found Pb concentrations ranging from 175.19 to 431.62 mg.Kg^{-1} in aphids that fed on peas growing in a contaminated environment with 0.5 mM of $Pb(NO_3)_2$ (equivalent to approximately 103.6 mg of Pb) in hydroponic cultivation.

The concentrations of Pb in the Lepidoptera samples ranged from 0.75 to 1.84 mg.Kg^{-1} (Fig. 1B). These values were higher than the concentrations found in the plant, indicating that lepidopterans are also capable of bioaccumulating Pb from kale and suggesting that biomagnification could occur. Previous studies have shown that lepidopteran larvae bioaccumulate Pb from artificial diets, exhibiting higher Pb concentrations than the food source (Zhou et al. 2012; Zhang et al. 2020). However, studies have shown that Pb bioaccumulation can be complex, and species-specific. Ali et al. (2019b) found that *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) larvae had a Pb concentration of 120 mg.Kg^{-1} regardless of whether they were fed a diet with low (50 mg.Kg^{-1}) or high (150 mg.Kg^{-1}) Pb concentration, whereas this result was attributed to the high elimination of Pb in the feces of these larvae (200 mg.Kg^{-1}). On the other hand, in the study by Zhang et al. (2020), when *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantriidae) larvae were fed an artificial diet containing 125 mg of Pb.Kg^{-1}, they had an average Pb concentration of 237 mg.Kg^{-1}. Another important factor is the life stage. The concentration of Pb tends to decrease in successive larval stages until reaching adulthood (Gintenreiter et al. 1993; Ortel 1995). Moreover, different parts of the body can accumulate different Pb concentrations. Zhang et al. (2020) found significantly higher Pb concentrations in the fat bodies, hemolymph, and alimentary canals than in the head and integument. Zhou et al. (2012) found Pb concentrations of up to 1,227 mg/kg in the midgut of *S. litura* larvae fed a diet with 100 mg of Pb.Kg^{-1}.

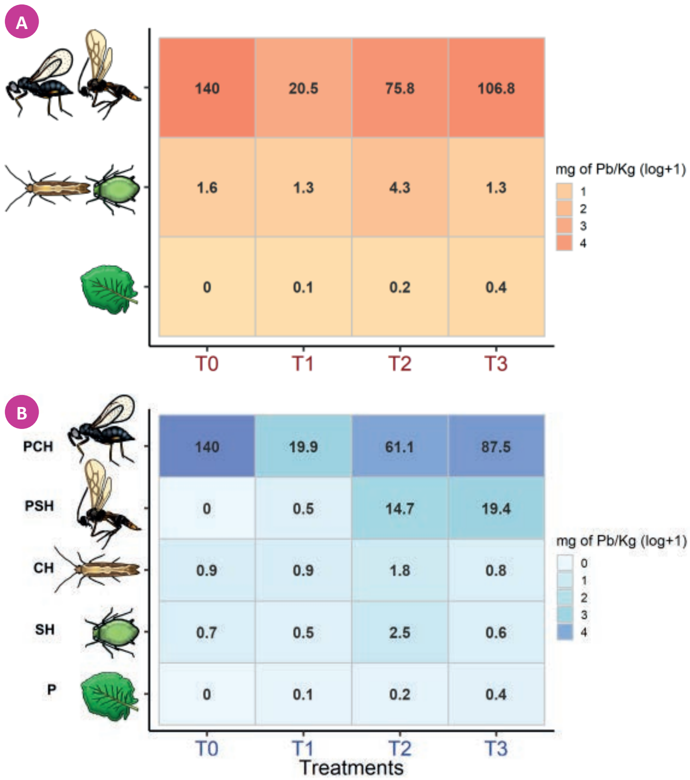

Figure 1. Heatmap showing the lead concentrations (mg.Kg^{-1}, based on dry weight) determined by ICP-OES. (A) Sum of concentrations by trophic level. (B) Concentration by functional group: P = plant, SH = sucking herbivores (aphids), CH = chewing herbivores (lepidopterans), PSH = parasitoids of sucking herbivores, and PCH = parasitoids of chewing herbivores. Colors represent Pb concentrations on a logarithmic scale. Numbers in the rectangles represent: the average concentrations (for P and SH) or the absolute concentrations of individual samples (for CH, PSH and PCH). T0 = control, T1 = 144, T2 = 360, and T3 = 600 mg of Pb(NO3)2/Kg of soil.

Concerning the impact of Pb on the biology of herbivorous insects, studies have demonstrated adverse effects even at lower concentrations. Woźniak et al. (2019) observed a decrease in the fecundity of the aphid *A. pisum* in individuals reared on peas treated with a hormone dose (0.075 mM $Pb(NO_3)_2$) (equivalent to about 15.54 mg of Pb) and a sublethal dose (0.5 mM $Pb(NO_3)_2$) (equivalent to about 103.6 mg of Pb). Furthermore, the treatment with the highest concentration of Pb decreased longevity, net reproduction rate, and changes in feeding behavior in individuals, prolonging the time required for aphids to reach the phloem and feed (Woźniak et al. 2019). For lepidopterans, Coleman et al. (2005) and Jhee et al. (2006) observed that Pb is toxic to *P. xylostella* larvae, at concentrations of 30, 17, 15, and 9.5 mg.Kg^{-1}, thereby causing a decrease in survival and pupation percentage in all treatments. Our experiment previously found adverse effects for herbivores at even lower concentrations. The population density of aphids and lepidopterans associated with kale was significantly reduced at mean concentrations of 0.388, 0.181, and 0.120 mg of Pb.Kg^{-1} of leaf (Morales-Silva et al. 2022). Studying the effect of HM on the biology of herbivorous insects is also important from the perspective of integrated pest management, as the exposure of key pests to Pb can increase their tolerance to insecticides (Zhou et al. 2012).

The parasitoid samples showed the highest concentrations of Pb, ranging from 0.54 to 140.00 mg of Pb.Kg^{-1} (Fig. 1A and B). This result indicates that aphid and lepidopteran parasitoids can accumulate Pb in much higher concentrations than their hosts, characterizing biomagnification in the kale-herbivores-parasitoids system. Studies on the bioaccumulation of Pb in parasitoids have mostly been limited to laboratory tests, revealing species-specific accumulation of this metal. In the study by Ortel (1995), adults of the parasitoid *Pimpla turionellae* (Linnaeus, 1758) (Hymenoptera: Ichneumonidae) accumulated higher concentrations of Pb than their host *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) when the latter was fed diets with Pb at 4, 43, and 430 mg.Kg^{-1}. The parasitoid/host concentration factors were 3-4 times higher than the host/food concentration factors, and adult parasitoids had an average Pb concentration of up to 9 mg.Kg^{-1}. On the other hand, in other species, a low accumulation of Pb was found compared to the host. For example, this was observed in *Glyptapanteles liparidis* (Bouché, 1834) (Hymenoptera: Braconidae), which were reared in *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae fed a diet with 4 and 20 mg of Pb.Kg^{-1} (Ortel et al. 1993). Similarly, in *Coptera occidentalis* Muesebeck, 1980 (Hymenoptera: Diapriidae) reared in pupae of *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), low accumulation of Pb was found when the pupae were raised on diets containing 100, 200, 400, and 800 mg of Pb.Kg^{-1} (Kazimirova et al. 1997; Kazimírová & Ortel 2000). The explanation found for the reduction in Pb accumulation in these parasitoids involves the greater elimination of the metal with host pupa's remains after the parasitoid's emergence (Kazimírová & Ortel 2000). The Pb concentrations in our parasitoid samples are the highest ever recorded, despite kale leaves having lower average Pb concentrations than all previously tested in laboratory studies. This result suggests that the bioaccumulation of Pb in parasitoids in the field may differ from what occurs in laboratory tests, being more intense.

Pb also negatively affects parasitoid biology. Ortel et al. (1993) observed a significant reduction in the number of hatched adults when *G. liparidis* developed within *L. dispar* larvae that were fed a diet contaminated with 4 mg of Pb.Kg^{-1}. In our experiment, we also previously observed this effect, despite the lower Pb concentrations in kale leaves. When compared to T0, T1 exhibited a twofold reduction, T2 a sixfold reduction, and T3 a sevenfold reduction in parasitoid population density. However, we did not observe a difference in the parasitism rate (Morales-Silva et al. 2022). This result demonstrates that the parasitoids cannot discriminate between contaminated and uncontaminated hosts; nevertheless, they experience a reduction in the number of individuals reaching adulthood due to Pb exposure (Morales-Silva et al. 2022). Thus, our findings underscore the potential impact of permitted Pb concentrations in Brazilian soils on ecosystem structure and functioning, as well as biological pest control (Morales-Silva et al. 2022; 2023).

Pb was detected in all insect samples, including T0, possibly due to shared greenhouse conditions. Winged aphid adults and lepidopterans could migrate between treatment plants, contaminating samples. Furthermore, studies suggest that metals like Pb could pass from parents to offspring in insects (Gintenreiter et al. 1993). Future fieldwork should address this by limiting insect migration and feeding and identifying transgenerational metal accumulation. This could involve infesting field plants with laboratory-reared insects and covering them with protective bags, for instance, while also monitoring the generations.

Despite limitations in our data, such as small sample numbers, our study sheds light on HM bioaccumulation in soil-plant-herbivore-parasitoid systems. Future research should use more insect samples or more sensitive metal detection techniques. Our findings highlight the need for more studies on HM bioaccumulation in natural ecosystems, as it may differ from laboratory conditions. We observed the highest concentrations of Pb in parasitoids, despite lower Pb levels in kale leaves compared to laboratory experiments. These results complement our previous findings showing the negative impact of Pb on species diversity, group abundance, and food web complexity in the same system (Morales-Silva et al. 2022; 2023). It emphasizes the necessity of revising environmental legislation concerning HM, addressing both ecological aspects such as ecosystem balance and species conservation, and applied aspects such as pest biological control.

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Authors' Contributions

TMS: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft preparation. BCS: Investigation, Data curation. LDBF: Conceptualization, Formal analysis, Writing - review & editing, Supervision. All authors read and approved the manuscript.

Conflict of Interest Statement

The authors declare no potential conflict of interest.

Supplementary Material

Supplementary data for this article be accessed at doi: https://doi.org/10.6084/m9.figshare.27625518.

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