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Responses of aphid and ladybird to lead transfer through soil and broad beans

Yan Li[1,], Shasha Wang[1,**], Huiru Si[1], Sijing Wan[1], Guoyong Li[2], Yinghua Shu[3], Xiaoyan Dai[4,5], Ruijuan Wang[4,5], Shigui Wang[1], Yifan Zhai[4,5,*], Can Li[2,*], Bin Tang[1,*]**

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang 311121, P.R. China
[2] Key Laboratory of Surveillance and Management of Invasive Alien Species in Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, Guiyang 550005, P.R. China
[3] Department of Ecology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, P.R. China
[4] Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan 250100, P.R. China
[5] Key Laboratory of Natural Enemies Insects, Ministry of Agriculture and Rural Affairs, Jinan 250100, P.R. China
* Corresponding authors: zyifan@tom.com; lican790108@163.com; tbzm611@hznu.edu.cn
** Authors contributed equally to this work

With 3 figures and 2 tables

Abstract: The impact of heavy metals on agricultural ecosystems has consistently been a topic of social concern. This study investigated the translocation of lead (Pb) along the soil – *Vicia faba* L. (Fabales: Fabaceae) – *Megoura crassicauda* (Hemiptera: Aphidoidea) – *Harmonia axyridis* (Coleoptera: Coccinellidae) system. Lead in soil could be transferred to broad bean, and the accumulation amount was highest in roots, followed by stems and leaves. Aphids and ladybirds could also accumulate lead through the food chain. Interestingly, the lead content of broad bean roots in the aphid feeding group was significantly higher than that in the non-aphid feeding group. Lead stress significantly reduced the germination rate and seedling height of broad bean, and inhibited root elongation. The expression levels of trehalase (*TRE*), trehalose-6-phosphate synthase (*TPS*), and vitellogenin (*Vg*), TRE activity, and carbohydrate content in aphids changed under lead treatment. The number of offspring produced by the second and fifth generation aphids was significantly reduced under lead treatment. Furthermore, high concentrations of lead treatment can prolong the development time of the 2nd and 3rd instar larvae of the ladybird. Feeding on aphids contaminated with lead can affect the survival of ladybirds. Our results further confirm the biological transfer of lead in the food chain and explore the adaptive mechanisms of aphids and ladybirds. These relevant results provide a theoretical basis for further exploring the molecular mechanism of lead homeostasis in plants and insects under lead stress.

Keywords: Food chain; Heavy metal pollution; Bioaccumulation; Trehalose metabolism; Development; Vitellogenin

1 Introduction

Soil is crucial for maintaining biodiversity and human survival, and its fertility and health directly affects the yield and quality of crops. However, soils around the world are currently facing threats including nutrient imbalance, salinization, and heavy metal pollution (Khan et al. 2021). Because soil microorganisms are unable to decompose heavy metals, there is a propensity for these metals to accumulate and be transformed into more toxic methyl compounds (Hu et al. 2023). It is noteworthy that heavy metals can be leached, which means the release of heavy metals from solid media (such as soil, slag, or waste) into liquids (such as surface water or groundwater) (Wang et al. 2024). Among heavy metals, lead (Pb) has been identified as the second most toxic metal following arsenic.

Heavy metal pollution in agroecosystems has emerged as a global concern. Survey data indicates that lead pollution in soil is prevalent globally (Kumar et al. 2015, 2020). Lead pollution in soil is a consequence of human activities including mining, metal smelting, transportation, sewage irrigation, and misuse of insecticides, as well as natural factors like volcanic eruptions and debris flows (Kumar et al. 2020). Lead not only exists in soils and sediments as a free metal ion, which can interact with inorganic molecules, but also exists in organic forms that bind with organic ligands like

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E. Schweizerbart'sche Verlagsbuchhandlung, 70176 Stuttgart, Germany, www.schweizerbart.de

amino acids, fulvic acid and humic acids (Vega et al. 2010; Kumar et al. 2020). Plants have the ability to uptake soluble lead from the soil via their roots, and they can also absorb lead compounds and particles from polluted air through the stomata on their leaves (Feng et al. 2019). In terrestrial ecosystems, herbivorous arthropods, comprising insects and other invertebrates, play a pivotal role in energy flow and nutrient cycling (Han et al. 2022). Therefore, lead can accumulate in primary consumers and then transfer to higher trophic level organisms within the food chain. Several studies have shown that predatory arthropods become contaminated with trace metals through the consumption of prey that has been exposed to contaminated plants (Sang et al. 2018; Naikoo et al. 2019). Furthermore, heavy metal pollution in agricultural soil poses a significant threat to food safety and human health.

As a significant natural enemy insect resource, the protection and utilization of *Harmonia axyridis* (Coleoptera: Coccinellidae) has attracted much attention. It has been demonstrated that zinc could be transferred along the broad bean – aphid – ladybird into the body of *H. axyridis* (Shi et al. 2020). To explore the potential transfer of lead to higher trophic level organisms along the food chain and its effects on plants and insects, we investigated the soil-*Vicia faba* L. (Fabales: Fabaceae)-*Megoura crassicauda* (Hemiptera: Aphidoidea)-*H. axyridis* system as our research model. We assessed the Pb concentration in organisms at various trophic levels. In addition, the responses of plants and insects to Pb treatment were investigated by monitoring the development of organisms and detecting the changes in energy metabolism.

2 Material and methods

2.1 Insect rearing

Aphids (*M. crassicauda*) were reared at 19 ± 1°C with 70 ± 5% humidity under a photoperiod of 14 h light: 10 h dark. The aphids were fed on broad bean seedlings. Ladybirds (*H. axyridis*) were reared at 25 ± 1°C with 70 ± 5% humidity under a photoperiod of 14 h light: 10 h dark. The ladybirds were fed on aphids.

2.2 Plant material

According to the studies of Zhang et al. (2017) and Naikoo et al. (2019), the concentrations of lead treatment were set at 12.5 (T1), 25 (T2), 50 (T3), 100 (T4), and 200 (T5) mg/L, respectively (Table S1). This experiment used tap water treatment as control (T0). Lead in the form of lead nitrate ($Pb(NO_3)_2$) was dissolved in tap water to make different concentrations of Pb^{2+} solution. To prevent the formation of $Pb(OH)_2$, nitric acid was added to make the solution acidic (pH = 6.0).

Broad beans were soaked in different concentrations of Pb^{2+} solution for 24 h, respectively, and then planted in soil (with a volume ratio of nutrient soil, vermiculite, and perlite of 6:2:1). About 43 broad beans were used per treatment. To prevent soil desiccation during the growth phase of beans, the beans would be watered with 400 mL of the corresponding concentration of solution every 3 days.

2.3 Experimental design

On the 10th day after planting, nearly 80 adult aphids devoid of heavy metal exposure (i.e., aphids that had fed on tap water-soaked and watered broad beans) were transferred onto the seedlings. The first generation of adult aphids (F1) were collected on the 10th day after transfer based on observations of the aphid developmental cycle. That is, adult aphids were collected on the 20th day after broad bean planting. Simultaneously, about 80 adult aphids were transferred to new broad bean seedlings to rear the second generation of aphids. The remaining aphids continued to reproduce on the original broad bean seedlings for heavy metal content detection. This process of aphid transfer and collection was continued to obtain the second (F2) third (F3), fourth (F4), and fifth (F5) generations of adult aphids.

2.4 Quantification of Pb concentration

Five broad bean seeds soaked for 24 h were collected per sample. To detect the transfer of lead along the soil – broad bean – aphid system, soils (1.0 g per sample), broad bean roots (40 plants per sample), stems (20 plants per sample), leaves (20 plants per sample) and aphids (all aphids at various developmental stages on the seedlings per sample) were collected on the 25th day after planting (the plants were watered eight times). Furthermore, to compare whether aphid feeding affects lead content in soil and broad beans, another batch of broad beans was prepared without transferring aphids to broad beans. Similarly, samples of soils, roots, stems, and leaves were collected on the 25th day after planting. Considering the susceptibility of young larvae to heavy metal stress and the ability of ladybirds to excrete heavy metals through pupal exuviae as reported in previous studies (Wang et al. 2017; Naikoo et al. 2019), we opted to transfer ladybird larvae on the first day of 3rd instar to broad bean seedlings with aphids. On the first day of pupal stage, about 30 ladybirds were collected per sample. Three repetitions were performed for each sample (N = 3). After all samples were collected, they dried in an oven at 60 °C and ground into a powder using a ceramic mortar. Then, all samples were sent to the Shiyanjia Lab (www.shiyanjia.com) for Pb concentration detection.

2.5 Determination of germination rate and the height of broad bean

We observed and recorded the germination of broad beans on the 8th, 10th, 12th, and 14th days after planting in the soil, and calculated the germination rate. About 43 broad beans were planted in each treatment, and 4 biological repeats were conducted for each group (N = 4). In addition, the heights

of broad bean seedlings were measured on the 8th, 10th, 12th, 14th, 16th, and 18th days after planting. Six repeats were conducted for each group (N = 6). Germination state and root morphology were recorded using a camera (EOS 50D, Canon, Japan) during the growth of broad beans.

2.6 Determination of carbohydrate content and trehalase activity

Trehalose plays important roles in the insect activities such as the energy metabolism, diapause and stress-resistances (Thorat et al. 2012). Trehalase (TRE) is a specificity enzyme which hydrolyses trehalose into two glucose molecules. Expression profile and enzyme activity of TRE are related to kinds of physiological processes of insects, so we measured the carbohydrate content, TRE activity in aphids. Fifteen adult aphids (each generation, from F1 to F5) were placed in a 1.5 mL centrifuge tube. The anthrone method was used to detect the trehalose content. Furthermore, the Glucose (GO) Assay Kit (GAGO20, Sigma, USA) was used to measure the glucose and glycogen content, as well as the activity of soluble trehalase (TRE1) and membrane-bound trehalase (TRE2). The protein content was detected by following the instructions provided in the BCA Protein Assay Kit (P0012, Beyotime, China). Three repetitions were performed for each treatment (N = 3).

2.7 Determination of the number of offspring produced by aphids

After each generation of adult aphids was transferred to a new broad bean seedling, the number of offspring produced was recorded. The broad bean seedlings were first numbered (from 1 to 8). Then two adult aphids were transferred to each broad bean seedling. The number of offspring produced by aphids was recorded daily. After counting, offspring were removed daily. The number of offspring produced by a single adult aphid within 7 days was calculated. Eight repeats were performed for each treatment (N = 8).

2.8 Real time fluorescence quantitative PCR (RT-qPCR)

To explore the effects of lead treatment on genes related to trehalose metabolism and reproduction in aphids, the expression levels of *TRE*, trehalose-6-phosphate synthase (TPS) gene, and vitellogenin (Vg) gene were measured. Ten adult aphids (each generation, from F1 to F5) were placed in a 1.5 mL centrifuge tube. Total RNA from aphids was extracted according to the manufacturer's instructions of the TRIzol reagent (Invitrogen, Carlsbad, California, USA). The first strand of cDNA was synthesized according to the instructions of the PrimeScript RT reagent kit with gDNA Eraser (Takara, Kyoto, Japan). The mRNA expression levels of *TRE*, *TPS*, and *Vg* (Table S2) were detected using TB Green ® The Premium Ex TaqTM (Tli RNaseH Plus) kit (Takara, Kyoto, Japan).

2.9 Observation and record of the development of ladybirds

Upon hatching from their eggs, the larvae were transferred into rearing boxes (plastic case, 3.7 cm × 3 cm × 3.3 cm). There were 15 larvae in each treatment. Single larvae were reared in a rearing box. Each ladybird was provided with a daily ration of approximately 150 aphids, which were fed on broad beans that had been treated with corresponding lead concentrations. To uphold hygienic conditions within the breeding environment, the excreta of the ladybirds were meticulously removed daily. The development status of ladybirds was observed every day, and the molting time was recorded to calculate the development time of each instar. Furthermore, ladybirds were weighed using a one-ten-thousandth balance (AL204, Mettler-toledo, Switzerland) within two hours post-molt. For documentation of any deformities during development, images of the ladybirds were captured utilizing a Leica EZ4 HD stereomicroscope in conjunction with the LAS EZ software. Survival rate of a certain instar = survival number of a certain instar/number of first instar larvae * 100%.

2.10 Data analysis

The T-test method in IBM SPSS Statistics 27 was used to analyze the differences between each lead treatment group and the control group, as well as between aphid feeding and non-feeding groups at the same treatment concentration. Additionally, Tukey's test was used to analyze the differences among the six groups. Finally, GraphPad Prism version 8.4.0 software was used to draw histograms.

3 Results

3.1 Lead concentration in soil, broad beans and insects under lead treatment

The lead concentration in broad bean seeds of the T1–T5 groups was significantly higher than that in the control group (0.31 ± 0.04 mg/kg) after soaking, up to 112.86–778.93 times, and there was a dose-dependent (T1: $F = 5.082$, t = −32.033, $P < 0.001$; T2: $F = 15.825$, t = −8.234, $P = 0.014$; T3: $F = 9.360$, t = −13.733, $P = 0.005$; T4: $F = 4.987$, t = −26.030, $P < 0.001$; T5: $F = 5.867$, t = −14.954, $P < 0.001$) (Fig. 1B). Lead content in soil and broad beans was tested at day 25 after sowing. Compared with the T0 group, the lead concentration in soil (N: $F_{5, 12} = 20498.152$; A: $F_{5, 12} = 21543.698$) and roots (N: $F_{5, 12} = 16733.952$; A: $F_{5, 12} = 22938.903$) of the T1–T5 groups significantly increased and there was a dose-dependent ($P < 0.001$ for ANOVA) (Fig. 1C and 1D). When there were no aphids feeding on broad beans, the lead concentration in stems ($F_{5, 12} = 7218.380$) and leaves ($F_{5, 12} = 2926.162$) of the T1–T5 groups was significantly higher than that of the control group ($P < 0.001$ for ANOVA) (Fig. 1E and 1F). However, when aphids fed on broad beans,

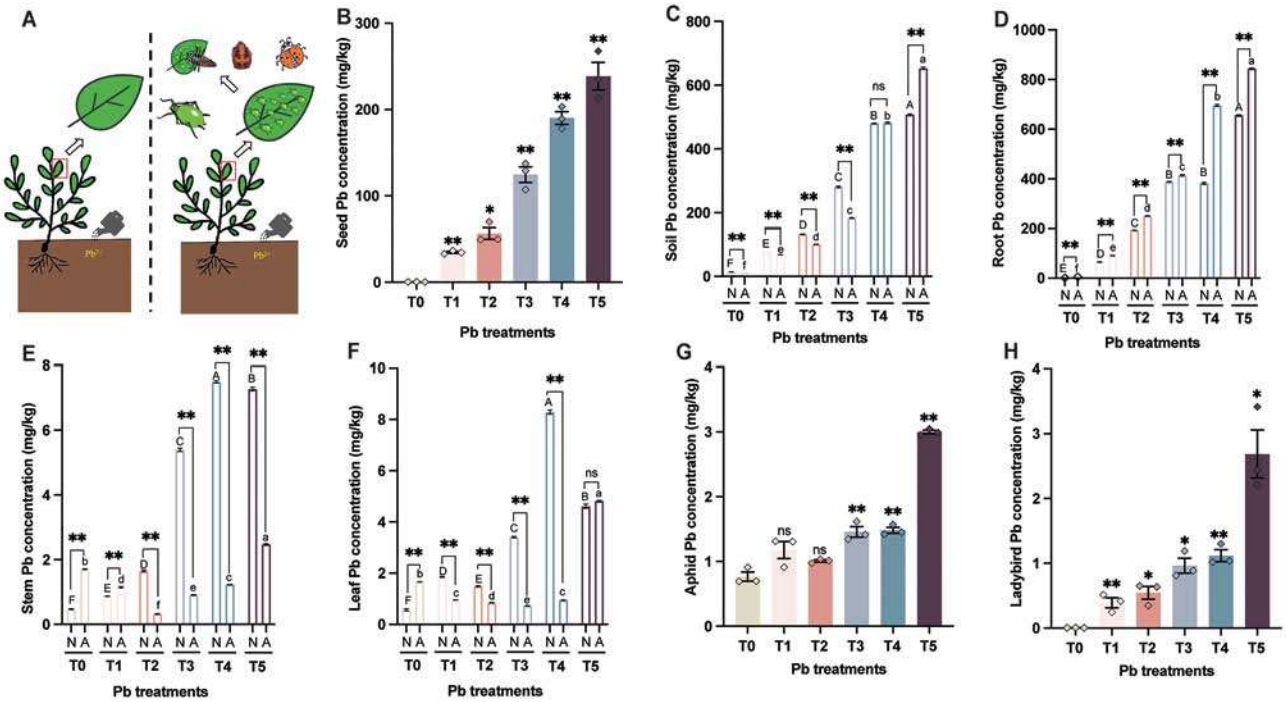

Fig. 1. Changes in lead (Pb) concentration in soil, broad beans, aphids, and ladybirds under lead treatment. **(A)** Schematic diagram of the soil-broad bean-aphid-ladybird model used in this study. **(B)** Pb concentration in broad bean seeds soaked for 24 h. Pb concentration in soil **(C)**, broad bean root **(D)**, stem **(E)**, and leaf **(F)** on the 25th day after planting. **(G)** Pb concentration in aphids. **(H)** Pb concentration in the pupa of *Harmonia axyridis*. (T0: tap water treatment, control group, T1: 12.5 mg/L, T2: 25 mg/L, T3: 50 mg/L, T4: 100 mg/L, T5: 200 mg/L. N: broad bean was not eaten by aphids; A: broad bean was eaten by aphids. Data are represented as mean ± SE. *: $P < 0.05$; **, $P < 0.01$ (T-test between control and treated group, as well as between aphid feeding and non-feeding groups at the same treatment concentration). Different lowercase or capital letters indicated significant differences among the treatments of soil, broad bean roots, stems, and leaves (Tukey's test, $P < 0.05$ level).

only the lead concentration in stems and leaves of the T5 (200 mg/L) group was significantly higher than the control group, while the concentration in T1–T4 groups was significantly lower than the control group ($F_{5, 12} = 2739.172$, $P < 0.001$ for ANOVA) (Fig. 1E and 1F). After aphid feeding, soil lead concentration in the T5 (200 mg/L) group increased significantly ($F = 0.091$, t = −35.262, $P < 0.001$), while that in the T1–T3 (12.5, 25, 50 mg/L) groups decreased significantly ($P < 0.001$) compared with that in the absence of aphids (T1–T3: $F = 0.012$, 0.113, 1.993; t = 28.716, 31.524, 38.635) (Fig. 1C). The lead concentration in roots of all groups (T0–T5) increased significantly ($P < 0.001$) compared to broad beans without aphids (T0–T5: $F = 0.509$, 0.001, 1.473, 4.111, 0.054, 0.023; t = −81.457, −21.173, −42.908, −8.622, −59.946, −60.327) (Fig. 1D). However, aphid feeding on broad bean significantly reduced lead concentration in stems (T2: $F = 2.244$, t = 40.938; T3: $F = 6.958$, t = 81.734; T4: $F = 3.617$, t = 175.700) and leaves (T2: $F = 5.677$, t = 25.102; T3: $F = 2.218$, t = 103.724; T4: $F = 4.703$, t = 88.705) in the T2–T4 treatments ($P < 0.001$) (Fig. 1E and 1F). This indicated that aphid feeding can have an impact on the accumulation of lead in soil and broad beans.

After aphids ingested lead from contaminated broad beans, the lead concentration in aphids of the T3–T5 (50, 100, 200 mg/L) groups was significantly higher than the control group (T3: $F = 0.066$, t = −6.388, $P = 0.003$; T4: $F = 1.395$, t = −8.424, $P = 0.001$; T5: $F = 5.146$, t = −29.253, $P < 0.001$) (Fig. 1G). Compared with the control group, the lead content in ladybird of the T1–T5 groups significantly increased (T1: $F = 6.774$, t = −4.939, $P = 0.008$; T2: $F = 15.234$, t = −5.475, $P = 0.032$; T3: $F = 11.762$, t = −8.313, $P = 0.014$; T4: $F = 14.964$, t = −12.109, $P = 0.007$; T5: $F = 12.330$, t = −7.245, $P = 0.019$) (Fig. 1H). The above results indicated that lead can transfer and accumulate along the soil – broad bean – aphid – ladybird.

3.2 Effects of lead treatment on the growth of broad bean

The germination rate of broad beans in the lead treatments (T1–T5) was significantly lower (df = 6, $P < 0.05$) than that in the control group at 8 (T1–T5: $F = 0.174$, 0.909, 1.841, 1.910, 1.197; t = 6.703, t = 5.873, 4.073, 3.136, 3.304), 10 (T1–T5: $F = 1.137$, 1.036, 0.071, 0.0003, 0.0004; t = 5.010, 3.945, 4.497, 3.712, 4.171), 12 (T1–T5: $F = 0.816$, 0.929,

0.300, 2.270, 0.006; t = 4.633, 2.952, 3.488, 3.953, 3.559) and 14 (T1–T5: F = 0.007, 0.118, 0.441, 2.087, 1.869; t = 5.723, 2.653, 4.344, 5.337, 3.690) days after planting, indicating that the germination of broad beans was significantly negatively affected (Fig. S1A). Moreover, compared with the control group, the length and number of lateral roots of broad bean in the lead treatments decreased (Fig. S1B and S1C), and the height of broad bean significantly decreased ($P < 0.05$) (Table S3). This indicated that heavy metal lead has a negative impact on the development and growth of broad bean roots.

3.3 Effects of lead treatment on trehalose metabolism in aphids

For glycogen, there was no significant difference in the content of the first to fifth generations of aphids fed on lead contaminated broad beans compared to the control group ($P > 0.05$) (Fig. S2). For trehalose, there was no significant difference in the content of aphids between the first and third generation lead treatment groups and the control group (Fig. S2A and S2C). In F2, the trehalose content of aphids in the T2 (25 mg/L) group significantly decreased compared to the control group ($F_{5, 12} = 4.135$, $P = 0.009$) (Fig. S2B). However, in F4 and F5, the trehalose content was significantly higher in the T2 (25 mg/L) group than in the control group ($P = 0.011$, 0.015) (Fig. S2D and S2E). Moreover, the trehalose content of the F4 aphids significantly increased in T1 (12.5 mg/L) ($P = 0.002$) and T3 (50 mg/L) ($P = 0.026$) groups (Fig. S2D). The glucose content of the F3 aphids significantly increased in T2 (25 mg/L) group compared to the T0 group ($P = 0.034$) (Fig. S2C). In F4, the glucose content in T1–T5 groups were significantly higher than that in the control group ($F_{5, 12} = 44.706$, $P < 0.001$) (Fig. S2D).

Under lead treatment, the TRE1 activity of the T1 (12.5 mg/L), T2 (25 mg/L) or T4 (100 mg/L) groups in F4 significantly increased ($F_{5, 12} = 6.686$, $P = 0.018$, 0.014, 0.002) (Fig. 2A). For the F3–F5 aphids, the TRE2 activity in the T1 (12.5 mg/L) and T2 (25 mg/L) groups was higher than that in the control group (Fig. 2B). Compared with the control group, *TRE* expression level of the F1 aphids in the T1–T5 groups significantly increased ($F_{5, 12} = 40.044$, $P < 0.001$), and only in T2 (25 mg/L) group, *TRE* expression level of the F3 aphids significantly increased ($P = 0.002$) (Fig. 2C). For the F1 aphids, the expression levels of *TPS* in the T1 ($P = 0.018$) and T3 ($P = 0.002$) groups were significantly higher than that in the control group (Fig. 2D). *TPS* expression level of the F2 and F3 aphids in the T2 (25 mg/L) group significantly increased ($P < 0.001$), while the expression level of the F4 aphids in the T5 (200 mg/L) group significantly decreased compared to the T0 group ($P = 0.026$) (Fig. 2D). The above results suggested that ingesting lead contaminated broad beans can have an impact on the trehalose metabolism of aphids, and there was no obvious rule among different generations of aphids.

3.4 Effects of lead treatment on aphid reproduction

For the F2 ($F_{5, 42} = 25.329$, $P < 0.001$), F3 ($F_{5, 42} = 4.908$, $P = 0.001$) and F5 ($F_{5, 42} = 16.279$, $P < 0.001$) aphids, the number of offspring produced by aphids in the T1–T4 groups significantly decreased compared to the control group (Fig. 3B, 3C and 3D). In particular, the number of offspring in the T5 (200 mg/L) group was significantly lower than that in the control group at the first ($P = 0.004$), second, fourth and fifth ($P < 0.001$) generations of adult aphid (Fig. 3A, 3B, 3D and 3E). In addition, the number of offspring produced by the F1 aphid in T2 (25 mg/L) group ($P = 0.001$) and the number of offspring produced by the F4 aphid in T1 (12.5 mg/L), T2 (25 mg/L) and T4 (100 mg/L) groups were significantly lower than those of the control group ($P < 0.001$) (Fig. 3A and 3D). Compared with the control group, the *Vg* expression levels of the F1 aphids in the T1–T5 groups significantly increased ($F = 13.877$, $P < 0.001$), but the expression level of the F5 aphids significantly decreased ($F = 7.475$, $P = 0.003$) (Fig. 3A and 3E). For the F3 and F4 aphids, the expression levels of *Vg* in T4 (100 mg/L) ($P = 0.011$, 0.045) and T5 (200 mg/L) ($P = 0.007$, 0.0003) groups were significantly lower than those of the control group (Fig. 3B–3D). The above results suggested that lead pollution inhibited aphid reproduction.

3.5 Effects of lead treatment on the development and survival of ladybirds

Although lead treatment did not affect the development time of the first larval stage, the fourth larval stage and the pupal stage ($P > 0.05$), the development time of the T3 (50 mg/L) ($P = 0.016$, 0.006) and T4 (100 mg/L) ($P = 0.003$, 0.001) groups was longer than that of the control group at the second and third larval stages (Table 1). When ladybirds fed on lead treated aphids, the weight of ladybirds at different developmental stages was not significantly different from that of the control group (Table 2). However, lead treatment had a significant impact on the ladybird survival, showed as a decrease, especially in the development of the larvae from the first to second instars (Fig. S3A). The abnormal phenotypes of ladybirds in the lead treatments were mainly difficult molting of larvae, failed eclosion, and deformed wings of adults (Fig. S3B).

4 Discussion

4.1 Transfer and accumulation of lead along soil-broad bean-aphid-ladybird

Once lead is released into the soil and infiltrates into plant roots, it can accumulate within the roots or be transferred to the aboveground parts of plants (Kumar et al. 2020). Moreover, research has found that lead mainly accumulates in plant roots (≥ 95%), with only a small portion transferred to the aboveground portion of the plant (Kiran & Prasad

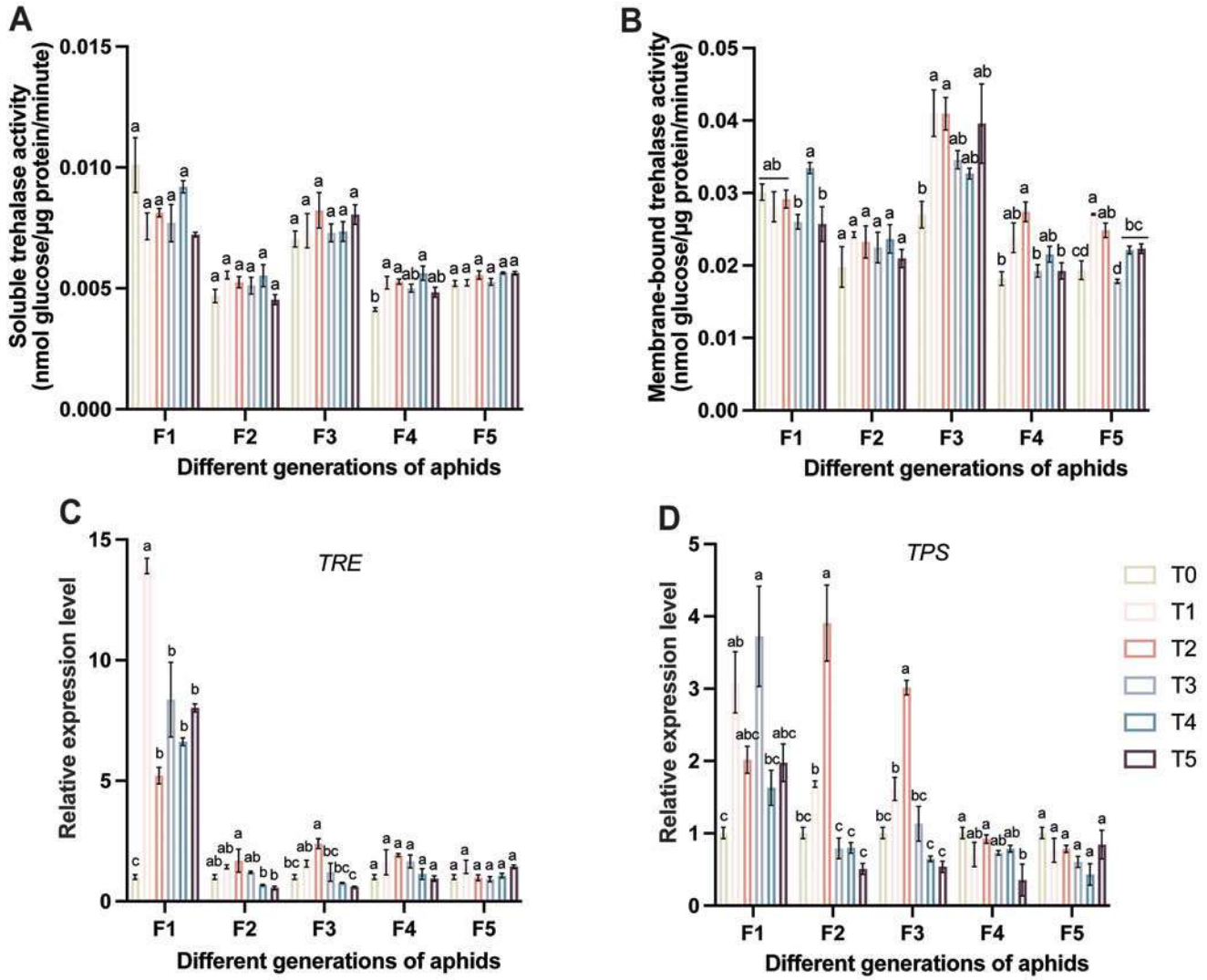

Fig. 2. Changes in the activity of two trehalase enzymes and the expression level of trehalase and trehalose-6-phosphate synthase genes in adult aphids of different generations under different lead concentrations. **(A)** Soluble trehalase activity. **(B)** Membrane-bound trehalase activity. **(C)** Trehalase gene, TRE. **(D)** Trehalose-6-phosphate synthase gene, TPS. (T0: tap water treatment, control group, T1: 12.5 mg/L, T2: 25 mg/L, T3: 50 mg/L, T4: 100 mg/L, T5: 200 mg/L). Data are represented as mean ± SE. Different letters indicated significant difference in enzyme activity or gene expression levels among different groups of the same generation (Tukey's test, $P < 0.05$ level).

2017). The results of this study were similar to previous findings (Fig. 1). There are multiple factors that contribute to hinder the movement of lead from roots to aboveground parts. First, negatively charged lignin and pectin are fixed or precipitated in the root cell wall, or bind to the carboxyl group of mucuronic acid in the cell wall (Arias et al. 2010). Secondly, insoluble Pb salts precipitate in the intercellular spaces of root cells (Zhang et al. 2017). Thirdly, the endoderm is a physical barrier to Pb translocation (Kumar et al. 2020). Fourth, Pb accumulates in the plasma membrane of root cells (Jiang & Liu 2010) or is isolated in the vacuoles of root skin cells and cortical cells (Kopittke et al. 2007).

Naikoo et al. (2019) and our research results confirm that lead can be transferred and accumulated from plants to higher trophic levels such as aphids and ladybirds (Fig. 1). However, unlike soil to root biomagnification, biological minimization of Pb occurs in the second trophic level of phytophagous insects and the third trophic level of predatory insects, with a transfer coefficient of < 1 (Zhang et al. 2017; Naikoo et al. 2019). It is speculated that it is related to the detoxification mechanism of insects, for example, aphids can discharge honey dew, and ladybirds can eliminate some harmful heavy metals through molting (Wang et al. 2017; Naikoo et al. 2019). Therefore, lead and other toxic metals

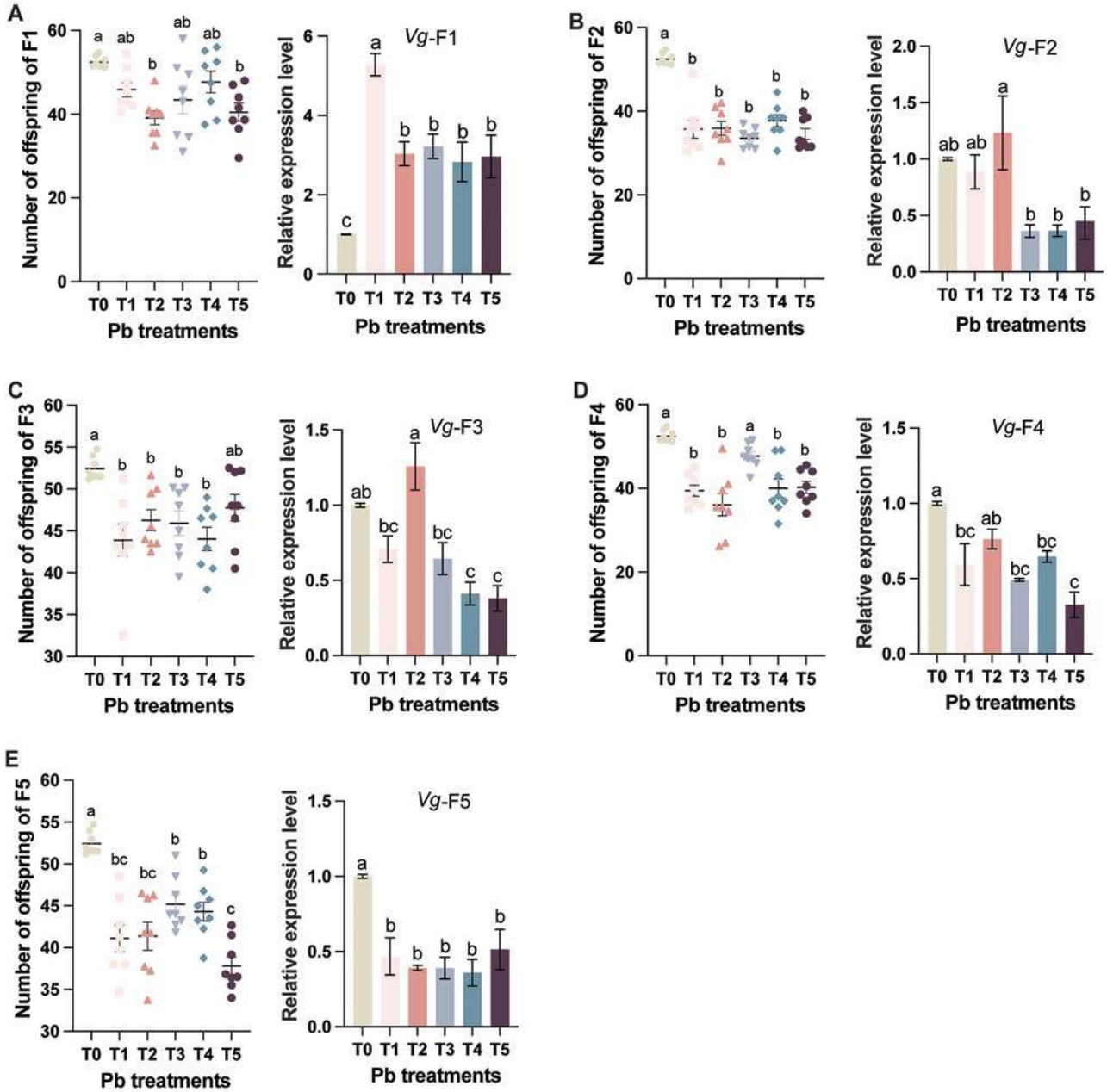

Fig. 3. Changes in the number of offspring and vitellogenin gene expression levels of adult aphids from different generations under different lead concentrations. The total number of offspring produced by a single adult aphid from the first generation **(A)** to the fifth generation **(E)** within 7 days and the expression level of vitellogenin (Vg) gene in the aphid on the first day of aphid production statistics. (T0: treated with tap water, as a control group, T1: 12.5 mg/L, T2: 25 mg/L, T3: 50 mg/L, T4: 100 mg/L, T5: 200 mg/L). Data are represented as mean ± SE. Different letters indicated significant difference in aphid production or gene expression levels among different groups of the same generation (Tukey's test, $P < 0.05$ level).

have fluidity and transmissibility in the food chain of agricultural insects (Naikoo et al. 2019; Wang et al. 2022).

Interestingly, this study found that the ingestion of broad bean by aphids affects the accumulation of lead in soil and broad bean (Fig. 1C–1F). In a similar study, the contents of Zn and Cd in the secretion of phloem increased after the plant was eaten by aphids (Stolpe et al. 2017a, 2017b). This suggests that aphids may contribute to the transfer of heavy metals from the soil to the plants through their feeding activities. However, more research is needed to fully understand the mechanisms by which aphids affect the uptake and storage of heavy metals in plants.

Table 1. Development duration of *Harmonia axyridis* under lead treatment (mean ± SE). Different lowercase letters indicated significant differences at the same developmental stage (Tukey's test, $P < 0.05$ level). I1: 1st Instar, I2: 2nd instar, I3: 3rd instar, I4: 4th instar, PP: prepupa, P: pupa, I-A: from 1st instar to adult, A: adult (lifespan).

Dev. stage	Development duration (day)						
	Control	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L	Statistics
I1	3.48 ± 0.16ab	3.47 ± 0.23ab	2.87 ± 0.12b	3.66 ± 0.21a	3.74 ± 0.20a	3.16 ± 0.08ab	$F_{5,52} = 2.98$, $P = 0.019$
I2	1.91 ± 0.08b	2.33 ± 0.14ab	2.29 ± 0.07ab	2.76 ± 0.16a	3.05 ± 0.35a	2.75 ± 0.23a	$F_{5,49} = 4.564$, $P = 0.002$
I3	2.23 ± 0.08b	2.95 ± 0.12ab	2.91 ± 0.11ab	3.26 ± 0.20a	3.63 ± 0.39a	2.98 ± 0.26ab	$F_{5,46} = 4.714$, $P = 0.001$
I4	4.23 ± 0.12a	5.21 ± 0.27a	5.15 ± 0.25a	4.99 ± 0.25a	5.09 ± 0.23a	5.19 ± 0.35a	$F_{5,40} = 2.203$, $P = 0.073$
PP	0.88 ± 0.07a	0.92 ± 0.05a	0.81 ± 0.09a	1.01 ± 0.03a	1.07 ± 0.17a	0.94 ± 0.02a	$F_{5,40} = 1.378$, $P = 0.253$
P	4.64 ± 0.08a	4.71 ± 0.06a	4.62 ± 0.09a	4.61 ± 0.07a	4.64 ± 0.11a	4.65 ± 0.13a	$F_{5,40} = 0.207$, $P = 0.957$
1-A	17.37 ± 0.32c	19.59 ± 0.55ab	18.61 ± 0.13bc	20.86 ± 0.42a	20.56 ± 0.51a	19.50 ± 0.56ab	$F_{5,40} = 8.412$, $P < 0.001$
A	52.00 ± 6.34a	43.75 ± 6.98a	35.37 ± 6.14a	47.14 ± 6.59a	52.60 ± 5.98a	56.83 ± 3.38a	$F_{5,36} = 1.517$, $P = 0.209$

Table 2. Weight of *Harmonia axyridis* under lead treatment (mean ± SE). Different lowercase letters indicated significant differences at the same developmental stage (Tukey's test, $P < 0.05$ level).

Developmental stage	Weight (mg)						
	Control	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L	Statistics
2nd instar	1.42 ± 0.25a	1.32 ± 0.11a	1.46 ± 0.07a	1.35 ± 0.07a	1.20 ± 0.07a	1.22±0.05a	$F_{5, 48} = 1.148$, $P = 0.348$
3rd instar	4.11 ± 0.20a	3.92 ± 0.16a	3.72 ± 0.15a	3.48 ± 0.20a	3.43 ± 0.28a	3.84 ± 0.33a	$F_{5, 48} = 1.376$, $P = 0.250$
4th instar	11.71 ± 0.49a	11.91 ± 0.76a	11.39 ± 0.63a	10.11 ± 0.60a	10.39 ± 1.11a	11.12 ± 0.85a	$F_{5, 46} = 1.093$, $P = 0.377$
Prepupa	34.26 ± 1.28a	30.62 ± 1.99a	30.71 ± 1.72a	28.23 ± 1.54a	30.46 ± 2.21a	31.42 ± 1.34a	$F_{5, 40} = 1.350$, $P = 0.264$
Pupa	32.46 ± 1.22a	28.19 ± 2.03a	28.39 ± 1.47a	26.44 ± 1.35a	27.74 ± 1.88a	29.18 ± 1.29a	$F_{5, 40} = 1.631$, $P = 0.174$
Adult	27.91 ± 1.11a	23.89 ± 1.79a	24.74 ± 1.36a	23.06 ± 1.11a	23.96 ± 1.58a	24.43 ± 1.30a	$F_{5, 40} = 1.439$, $P = 0.231$

4.2 Inhibition of root elongation and seed germination is a significant feature of lead poisoning in plants

Lead is a non-essential element for plant growth and development, so excessive lead can have detrimental effects on plants, including impaired chlorophyll production, cell division, root elongation, chloroplast lamellar tissue, plant growth, seed germination, seedling development, and transpiration (Zulfiqar et al. 2019). Our results are consistent with previous studies indicating that lead stress inhibits broad bean germination, root elongation, and plant growth (Fig. S1 and Table S3).

Excessive lead contamination can impede seed germination in rice, barley, corn, lentils, and alfalfa (Sedzik et al. 2015; Zhang et al. 2018). The reason is that lead affects seed physiology and morphology (Seneviratne et al. 2019). Specifically, lead can interfere with enzymes involved in seed germination, delaying the emergence of radicles by affecting the activities of polyphenol oxidase and peroxidase, reducing the activities of enzymes involved in carbohydrates, and increasing the content of carbohydrates and proteins (Sethy & Ghosh 2013), thereby affecting the oxidative capacity of roots (Singh et al. 2011). Liu et al. (2008) also discovered that lead pollution affects the growth of plant roots. This

phenomenon has also been observed in cowpea (Kopittke et al. 2007) and *Prosopis* sp. (Arias et al. 2010). The reason is that lead exposure hampers root growth by damaging biological membranes and impacting the structure of organelles within root meristematic cells (Jiang & Liu 2010). Lead contamination also impairs early plant growth, which might be connected with impaired nutrient metabolism, plant water relations and photosynthesis (Zulfiqar et al. 2019).

4.3 Effects of lead on development and energy metabolism of phytophagous and predatory insects

Heavy metals absorbed by insects have significant impacts on their growth rate, survival, and other physiological aspects (van Ooik et al. 2007). The results of this study showed that the number of offspring reduced (Fig. 3), the development time of larvae in the high concentration treatment group was prolonged from the 2nd to 4th instar stages (Table 1) and the survival rate decreased (Fig. S3). Similarly, when *Tenebrio molitor* were fed diets containing Pb, their survival and total live weight decreased (van der Fels-Klerx et al. 2016). Interestingly, in *Coccinella transversalis*, the weight of newly emerged adults in the high-dose Pb treatments decreased (Naikoo et al. 2019), while cadmium (Cd) treatment had no effect (Naikoo et al. 2021). But lead had no significant impact on the weight of ladybirds here (Table 2). The weight measured in this study did not take into account the inherent differences between individuals. Weight gain, that is, the difference in body weight of the same insect at different ages, is a more meaningful parameter to detect weight change, as reported by Shi et al. (2020). In addition, demographic-based study would be needed in further studies to assess effects of lead on aphids and ladybirds (Chi et al. 2022).

Trehalose plays a multifaceted role in insects' physiological activities (Thorat et al. 2012). Yu et al. (2020) found that under long-term Cd stress, the trehalose content of *Aedes albopictus* increased, while the glucose content and trehalase activity decreased. Moreover, trehalose metabolism in *A. albopictus* is also affected under acute Cd stress (Yu et al. 2019). When *Spodoptera litura* ingested artificial diets supplemented with heavy metal Cd, the trehalose content in the adults decreased, and the activities of trehalose synthase and trehalose-6-phosphate synthase were also affected (Yang 2021). In summary, insects can resist heavy metal stress by regulating trehalose metabolism (Fig. S2 and Fig. 2). Further research is needed to understand the specific molecular mechanisms in insects that adapt to and regulate heavy metal stress.

From the above discussion, it is clear that heavy metal pollution has a profound impact on agroecosystem. This pollution not only hinders the normal growth of crops, but more seriously, these heavy metals gradually accumulate in agricultural products. Once these contaminated agricultural products enter the human body through the food chain, they pose a potential and serious threat to the health of humans. To maintain the stability of agroecosystem and human health, we must take practical and effective measures to reduce the emission and accumulation of heavy metals. Meanwhile, we also need to actively carry out soil remediation and ecological restoration work.

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Supplementary material and methods, Table S1–S3, Figure S1–S3