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The effects of lead (Pb) and pest damage on soil enzyme activities, pakchoi and *Spodoptera litura* performance

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Abstract

Plant–soil interactions have bottom–up and top–down effects within a plant community. Heavy metal pollution can change plant–soil interactions, directly influence bottom–up effects and indirectly affect herbivores within the community. In turn, herbivores can affect plant–soil interactions through top–down effects. However, the combined effects of heavy metals and herbivores on soil enzymes, plants and herbivores have rarely been reported. Therefore, the effects of lead (Pb), *Spodoptera litura* and their combined effects on soil enzyme activities, pakchoi nutrition, defence compounds and *S. litura* fitness were examined here. Results showed that Pb, *S. litura* and their combined effects significantly affected soil enzymes, pakchoi and *S. litura*. Specifically, exposure to double stress (Pb and *S. litura*) decreased soil urease, phosphatase and sucrase activities compared with controls. Furthermore, the soluble protein and sugar contents of pakchoi decreased, and the trypsin inhibitor content and antioxidant enzyme activity increased. Finally, the *S. litura* development period was extended, and survival, emergence rates and body weight decreased after exposure to double stress. The combined stress of Pb and *S. litura* significantly decreased soil enzyme activities. Heavy metal accumulation in plants may create a superposition or synergistic effect with heavy metal-mediated plant chemical defence, further suppressing herbivore development. Pb, *S. litura* and their combined effects inhibited soil enzyme activities, improved pakchoi resistance and reduced *S. litura* development. The results reveal details of soil–plant–herbivore interactions and provide a reference for crop pest control management in the presence of heavy metal pollution.

Introduction

Soil provides moisture and nutrients to plants, which can alter the growth and nutritional quality of plant shoot systems (Wang *et al*., 2019; Han *et al*., 2022). These changes of plant shoot systems can subsequently affect the performance of foliar herbivores, thereby influencing the ecological dynamics via bottom–up effects (Pineda *et al*., 2010; Wang *et al*., 2019; Han *et al*., 2022). Plants produce vegetative litter and rhizospheric secretions that alter the physicochemical properties and microbial communities of the soil (Orwin *et al*., 2010; Cantarel *et al*., 2015; Carrillo *et al*., 2019; Huberty *et al*., 2020, 2022). Furthermore, herbivorous insects affect the concentrations of primary and secondary plant root tissue compounds, which can directly affect interactions between the roots and soil organisms; therefore, top–down effects, such as plant–herbivore interactions, can impact plant–soil interactions (Martijn *et al*., 2013; Huberty *et al*., 2020; Han *et al*., 2022).

Heavy metal pollution seriously impacts global agricultural ecosystems (Abdu *et al*., 2017; Lin *et al*., 2022a). Soil enzymes play a decisive role in metabolic processes and the overall balance of soil (Nurzhan *et al*., 2022; Tan *et al*., 2023). Heavy metals can influence soil enzyme activities, although the degree of influence depends on different factors, such as heavy metal type and concentration, and soil enzyme type (Gao *et al*., 2010; Aponte *et al*., 2020; Tang *et al*., 2022). Herbivore feeding can also affect the production of plant root secretions and indirectly affect soil enzyme activity (Classen *et al*., 2006; Huang *et al*., 2013; Song *et al*., 2015; Hoysted *et al*., 2018; Long *et al*., 2022).

Following damage by herbivores, a plant may respond by exhibiting induced resistance through long-distance signal conduction and integration (Karban, 2011; Li, 2016). The formation of herbivore-induced compounds requires certain nutrients and energy, which ultimately reduces the plant's nutrient content (Karban and Baldwin, 1997; Zavala *et al*., 2004; Dicke, 2015; Li, 2016). Herbivore-induced plant compounds include defence proteins and toxic secondary metabolites (Paudel *et al*., 2019; Li *et al*., 2021). These compounds destroy digestion and the absorption system of herbivorous insects, subsequently interfering with nutrient

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absorption and utilisation by insects (Li, 2016; Zhao *et al.*, 2019; Li *et al.*, 2021). This is detrimental to the developmental performance of insects and may result in their death (Karban and Baldwin, 1997; Paudel *et al.*, 2019; Zhao *et al.*, 2019; Li *et al.*, 2021). For example, when *Helicoverpa zea* consumes tomatoes, the chemical content of the leaf increases together with an increase in the trypsin protease inhibitor (TPI) content (Paudel *et al.*, 2019). TPI can inhibit the digestion, absorption and utilisation of food proteins by insects (Bhattacharyya *et al.*, 2007; Li, 2016; Zhao *et al.*, 2019). Herbivore-induced plant chemical defences significantly alter plant sugars and nitrogenous compounds (Watanabe and Kitagawa, 2000; Li *et al.*, 2008; Long *et al.*, 2022); therefore, plant-induced resistance inhibits herbivorous insects from acquiring nutrients from plants (Hu, 2004; Li *et al.*, 2008; Li, 2016).

Heavy metals are transferred and enriched through the soil–plant–herbivorous insect food chain and affect the plant nutrient content by activating plant antioxidant enzyme systems and causing plant resistance (Jiang *et al.*, 2018, 2020; Sang *et al.*, 2018; Tibbett *et al.*, 2021). The antioxidant enzyme system in plants protects them from heavy metal damage (Bhaduri and Fulekar, 2012; Sidhu *et al.*, 2018). For example, the nutrient content of *Chenopodium murale* decreases when the lead (Pb) content is excessively high, whereas the activities of superoxide dismutase (SOD), polyphenol oxidase (POD), and catalase (CAT) significantly increase (Sidhu *et al.*, 2018).

Heavy metals trigger bottom–up effects and affect pest populations (Butler and Trumble, 2008; Han *et al.*, 2022). Therefore, herbivores feeding on heavy metal-stressed plants are not only subject to the direct toxic effects of the heavy metal(s) (elemental defence) but also to a possible heavy metal-mediated plant defence response (chemical defence) (Boyd, 2012; Sahu *et al.*, 2018; Chen *et al.*, 2020; Jiang *et al.*, 2020; Yactayo-Chang *et al.*, 2020; Lin *et al.*, 2022b). Insect larvae usually show adverse effects, such as weight loss; they undergo a prolonged development period and have a decreased emergence rate (Chen *et al.*, 2020; Jiang *et al.*, 2021; Lin *et al.*, 2022b). Heavy metal accumulation in plants and the heavy-metal-mediated plant defence response have a superimposed or synergistic effect that inhibits insect growth and development (Boyd, 2012; Cheruiyot *et al.*, 2015; Sahu *et al.*, 2018; Chen *et al.*, 2020; Jiang *et al.*, 2020; Yactayo-Chang *et al.*, 2020; Lin *et al.*, 2022b). Heavy-metal-driven plant defence mechanisms are inconsistent with herbivore-induced defence mechanisms; however, their effects on herbivores are similar when inducing plant defence expression (Sidhu *et al.*, 2018; Zhang *et al.*, 2022). Therefore, these two mechanisms simultaneously regulate plant defences against herbivores (Huang *et al.*, 2021).

Plant–soil interactions have bottom–up and top–down effects (Huberty *et al.*, 2020, 2022; Han *et al.*, 2022). For example, high concentrations of cadmium (Cd) or copper (Cu) in contaminated soil enhance maize resistance to *Spodoptera frugiperda* and significantly prolong its larval development period (Winter *et al.*, 2012). Soil urease and phosphatase activities decrease following soybean consumption by *S. litura* (Long *et al.*, 2022). Several studies have shown that heavy metal and herbivore damage can induce bottom–up and top–down effects, respectively; however, the combined effects of heavy metals and herbivores on soil, plants and herbivorous insects have rarely been studied.

This study investigated the bottom–up effects triggered by Pb, top–down effects triggered by *S. litura*, and combined effects of Pb and *S. litura* on the soil enzyme activity and fitness of pakchoi and *S. litura*. The results reveal details of soil–plant–herbivore interactions and provide a reference for crop pest control management in the presence of heavy metal pollution.

Materials and methods

Soil and plants

The soils used in this study were collected at a depth of 0–20 cm in a fallow field within a suburb of Shaoyang City, China (26.9°N, 111.3°E). The soil was of medium fertility, with a pH of 6.13 and a Pb content of 4.02 mg kg^{-1}. The total nitrogen, total phosphorus, total potassium and organic matter contents were 1.25, 0.52, 21.08 and 20.23 g kg^{-1}, respectively, and the alkali-hydrolytic nitrogen, available phosphorus and available potassium contents were 113.81, 6.05 and 155.28 mg kg^{-1}, respectively. The soils were air-dried and filtered through a 20-mesh sieve. $Pb(NO_3)_2$ was then dissolved in tap water and sprayed evenly on the soil at a concentration of 400 mg kg^{-1} (dry weight: $Pb[NO_3]_2$/soil). The Pb-treated soil was aged for 15 days. Control soil was treated with tap water and aged for 15 days.

Pakchoi ('Shouhe') seeds were sown in both treated and controlled soils. Following germination, robust seedlings were selected for transplantation into plastic pots (diameter: 20 cm, height: 18 cm) filled with either treated or control soils. Each pot was planted with one seedling. Pakchoi seedlings were planted in sequential batches every 5 days from 10 May to 20 June 2022. To ensure an adequate number of plants, each treatment batch was grown in 24 pots. Pots were covered with gauze netting to prevent infestation by other organisms. The plants were maintained in the field and were used in the experiments after they reached the six-leaf stage.

Herbivores

In 2021, *S. litura* individuals were collected from tobacco fields in the suburbs of Shaoyang City. Larvae were reared on artificial diets in incubators at 26 ± 1 °C, 60 ± 10% relative humidity and with a 14:10 (light: dark) photoperiod. Adult moths were supplied with 10% honey solution on a cotton ball, which was refreshed daily.

Experimental setup

Four treatments were set up in this experiment as follows: pakchoi plants planted in control soil, without any treatment (CK group); pakchoi plants planted in Pb-treated soil (Pb stress group); pakchoi plants planted in control soil with three 2nd instar *S. litura* larvae placed on each leaf (the 2nd–5th leaf from the bottom of the pakchoi plant). When approximately 1/3rd of the leaf area was consumed, the larvae were removed from the plants (herbivore treatment group). In addition, pakchoi plants were planted in Pb-treated soil and three 2nd instar *S. litura* larvae were placed on each leaf (the 2nd–5th leaf from the bottom of the pakchoi plant). When approximately 1/3rd of the leaf area was consumed, the larvae were removed from the plants (double stress group, Pb + herbivore). Each pakchoi plant was used in experiments immediately after treatment.

Ten pakchoi plants were randomly selected from each treatment. Subsequently, 10 g (wet weight) of rhizosphere soil (0–1 cm outside the pakchoi root system) was collected from each pakchoi plant and air-dried separately to measure phosphatase activity (disodium phenyl phosphate colorimetric method), urease (sodium phenate-sodium hypochlorite colorimetric method) and sucrose

(3,5-dinitrosalicylic acid colorimetric method) ($n = 10$ per treatment) (Guan, 1986).

Tissue samples from the 3rd leaf (from the top) of each pakchoi plant ($n = 10$ per treatment) were flash-frozen in liquid nitrogen. The samples were then stored at −80 °C until further analysis. Soluble protein, soluble sugar, Pb and TPI contents were determined by Coomassie brilliant blue staining, the anthrone method, an atomic absorption spectrophotometer (AA-6880; Shimadzu) and the spectrophotometric method (DR6000; Hach), respectively (Bradford, 1976; Li, 2016; Zhang *et al*., 2020; Winiarska-Mieczan *et al*., 2023).

POD, SOD and CAT activities were determined using guaiacol, nitroblue tetrazolium photoreduction and potassium permanganate titration methods, respectively (Chance and Maehly, 1955; Gupta *et al*., 1993).

Two neonate *S. litura* were raised in an insect-rearing box (diameter: 5 cm, height: 3 cm) containing moisturising filter paper and provided with fresh leaves excised from treated plants daily (CK group, Pb stress group, herbivore treatment group and double stress group, respectively). The neonate larvae in each treatment group were counted, and the pupated larvae were regarded as one repetition ($n = 30$ per treatment). The larval survival rate (30/total tested larvae per treatment), developmental period (from newly hatched larvae to pupae), emergence rate and adult dry weight (measured after adults had been dried in an 80 °C thermostatic drying box for 48 h) were recorded.

Data analysis

Data were checked for homogeneity of variance, and soluble sugar content and *S. litura* adult dry weight were logarithmically transformed. Two-way analysis of variance (ANOVA) was used to determine the effects of Pb stress and *S. litura* feeding on soil enzyme activities and the fitness of pakchoi and *S. litura*. A logistic generalised linear model was used for the analysis because the survival and emergence rates of *S. litura* were presented as binary data. Multiple comparisons were performed using Tukey's honestly significant difference test. All analyses were performed using R (R Development Core Team, 2022).

Results

Soil enzyme activities

Soil urease activity was significantly affected by Pb stress ($F_{1, 56} = 98.22$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 27.83$, $P < 0.05$) and their combined effects ($F_{1, 56} = 4.24$, $P < 0.05$). Compared with the controls, soil urease activity was the lowest in the double stress group (Pb + herbivore group, 47.86% decrease), followed by the Pb stress group (34.19% decrease) and then the herbivore treatment group (21.37% decrease). There was a significant difference among treatments ($P < 0.05$) (fig. 1a).

Soil phosphatase activity was significantly affected by Pb stress ($F_{1, 56} = 578.65$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 78.21$, $P < 0.05$) and their combined effects ($F_{1, 56} = 4.62$, $P < 0.05$). Compared with the controls, soil phosphatase activity was the lowest in the double stress group (46.81% decrease), followed by the Pb stress group (31.21% decrease) and then the herbivore treatment group (9.57% decrease). There was a significant difference among treatments ($P < 0.05$) (fig. 1b).

Soil sucrase activity was significantly affected by Pb stress ($F_{1,56} = 151.72$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 68.12$, $P < 0.05$) and their combined effects ($F_{1, 56} = 1.90$, $P < 0.05$). Compared with the controls, soil sucrase activity was the lowest in the double stress group (36.93% decrease), followed by the Pb stress group (19.59% decrease) and then the herbivore treatment group (12.36% decrease). There were significant differences among treatments ($P < 0.05$) (fig. 1c).

Pakchoi analysis

The soluble protein content was significantly affected by Pb stress ($F_{1, 56} = 7.42$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 12.22$, $P < 0.05$) and their combined effects ($F_{1, 56} = 5.24$, $P < 0.05$). Compared with that in the controls, the soluble protein content was the lowest in the double stress group (45.05% decrease), followed by the herbivore treatment group (31.53% decrease) and then the Pb stress group (27.03% decrease). There was a significant difference between the double stress group and the other treatment groups ($P < 0.05$) (fig. 2a).

The soluble sugar content was significantly affected by Pb stress ($F_{1, 56} = 31.53$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 27.25$, $P < 0.05$) and their combined effects ($F_{1, 56} = 8.75$, $P < 0.05$). Compared with that in the controls, the soluble sugar content was the lowest in the double stress group (41.46% decrease), followed by the Pb stress group (16.03% decrease) and then the herbivore treatment group (14.52% decrease). There was a significant difference between the double stress group and the other treatment groups ($P < 0.05$) (fig. 2b).

TPI content was significantly affected by Pb stress ($F_{1, 56} = 177.64$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 333.39$, $P < 0.05$) and their combined effects ($F_{1, 56} = 21.23$, $P < 0.05$). Compared with that in the controls, the TPI content was the highest in the double stress group (1.03-fold increase), followed by the herbivore treatment group (0.75-fold increase) and then the Pb stress group (0.59-fold increase). There were significant differences among treatments ($P < 0.05$) (fig. 2c).

POD activity was significantly affected by Pb stress ($F_{1, 56} = 146.14$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 109.22$, $P < 0.05$) and their combined effects ($F_{1, 56} = 8.40$, $P < 0.05$). Compared with that in the controls, POD activity was the highest in the double stress group (3.25-fold increase), followed by the Pb stress group (1.33-fold increase) and then the herbivore treatment group (1.09-fold increase). There was no significant difference between the Pb and herbivore stress groups ($P > 0.05$) (fig. 2d).

SOD activity was significantly affected by Pb stress ($F_{1, 56} = 111.28$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 61.98$, $P < 0.05$) and their combined effects ($F_{1, 56} = 17.03$, $P < 0.05$). Compared with that in the controls, SOD activity was the highest in the double stress group (0.88-fold increase), followed by the Pb stress group (0.31-fold increase) and then the herbivore treatment group (0.18-fold increase). There was no significant difference between the Pb and herbivore stress groups ($P > 0.05$) (fig. 2e).

CAT activity was significantly affected by Pb stress ($F_{1, 56} = 149.62$, $P < 0.05$), herbivore treatment ($F_{1, 56} = 109.80$, $P < 0.05$) and their interaction ($F_{1, 56} = 16.20$, $P < 0.05$). Compared with that in the controls, CAT activity was the highest in the double stress group (1.73-fold increase), followed by the Pb stress group (0.63-fold increase) and then the herbivore treatment group (0.49-fold increase). There was no significant difference between the Pb and herbivore stress groups ($P > 0.05$) (fig. 2f).

The leaf Pb content was significantly affected by Pb stress ($F_{1, 56} = 479.24$, $P < 0.05$) and herbivore treatment ($F_{1, 56} = 0.048$, $P < 0.05$); however, there was no effect from their combined

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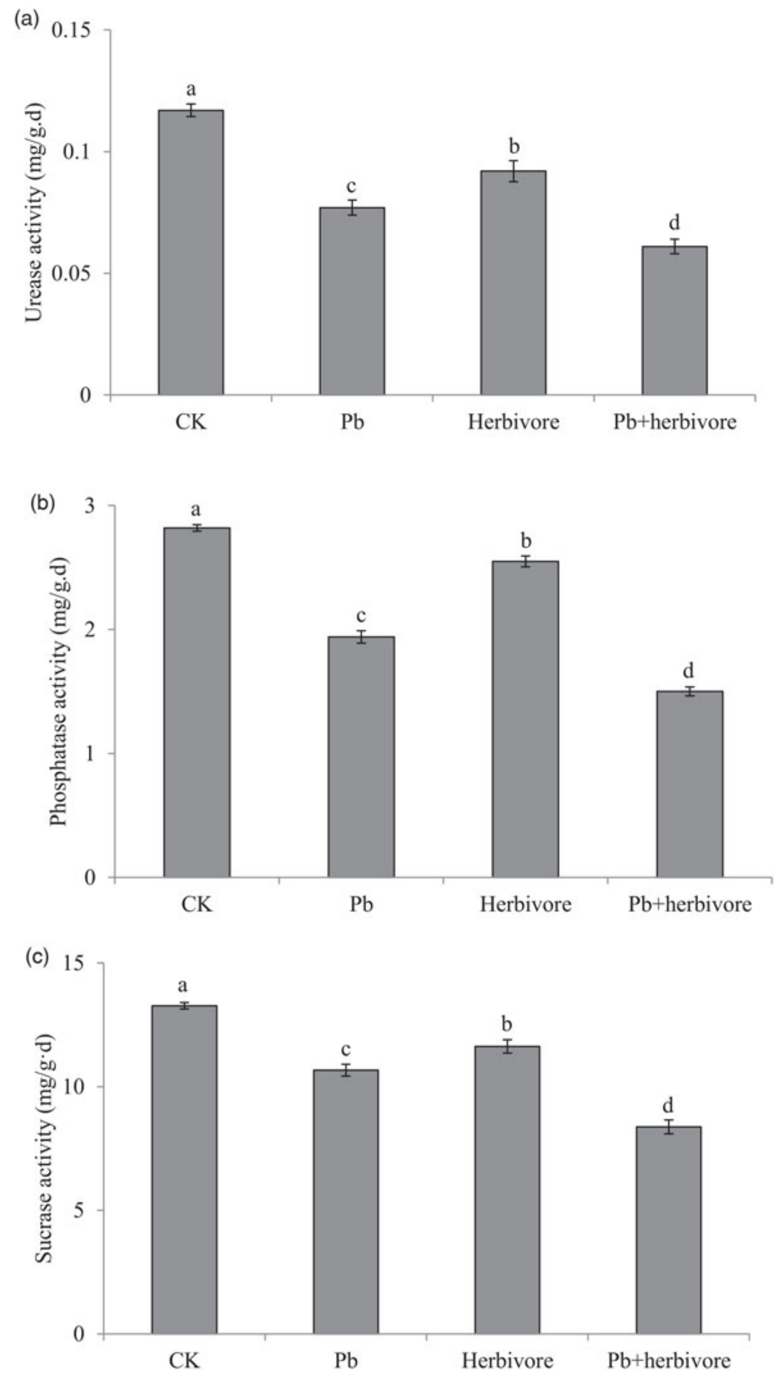

Figure 1. (a) Urease, (b) phosphatase and (c) sucrase activities of soil. Soil enzyme activity under Pb stress, herbivore stress (*Spodoptera litura*), Pb and herbivore double stress and the control (CK). Data are mean values ± standard error, and different lowercase letters indicate statistically significant differences as determined using Tukey's honestly significant difference.

effects ($F_{1,56} = 0.07$, $P > 0.05$). The herbivore treatment group had the lowest leaf Pb content ($5.6 \times 10^{-3} \pm 4.56 \times 10^{-4}$ mg kg^{-1}), and the double stress group had the highest leaf Pb content (0.37 ± 0.03 mg kg^{-1}), with a significant difference ($P < 0.05$) (fig. 2g).

Herbivore performance

The *S. litura* survival rate was significantly affected by Pb stress (logistic ANOVA, $\chi^2 = 12.47$, $P < 0.05$), herbivore treatment (logistic ANOVA, $\chi^2 = 9.42$, $P < 0.05$) and their combined effects (logistic ANOVA, $\chi^2 = 1.84$, $P < 0.05$). Compared with that in the controls, survival rates decreased by 33.7, 29.69 and 49.36% in the Pb, herbivore and double stress groups, respectively. There was no significant difference between the Pb and herbivore treatment groups ($P > 0.05$) (fig. 3a).

The larval development time was significantly affected by Pb stress ($F_{1,116} = 408.03$, $P < 0.05$), herbivore treatment ($F_{1,116} = 234.44$, $P < 0.05$) and their combined effects ($F_{1,116} = 22.64$, $P < 0.05$). Compared with that in the controls, the larval development time was prolonged by 3.85, 2.63 and 8.86 days in the Pb, herbivore and double stress groups, respectively. There were significant differences among treatments ($P < 0.05$) (fig. 3b).

The *S. litura* emergence rate was significantly affected by Pb stress (logistic ANOVA, $\chi^2 = 8.22$, $P < 0.05$), herbivore treatment (logistic ANOVA, $\chi^2 = 4.29$, $P < 0.05$) and their combined effects (logistic ANOVA, $\chi^2 = 0.12$, $P < 0.05$). Compared with that in

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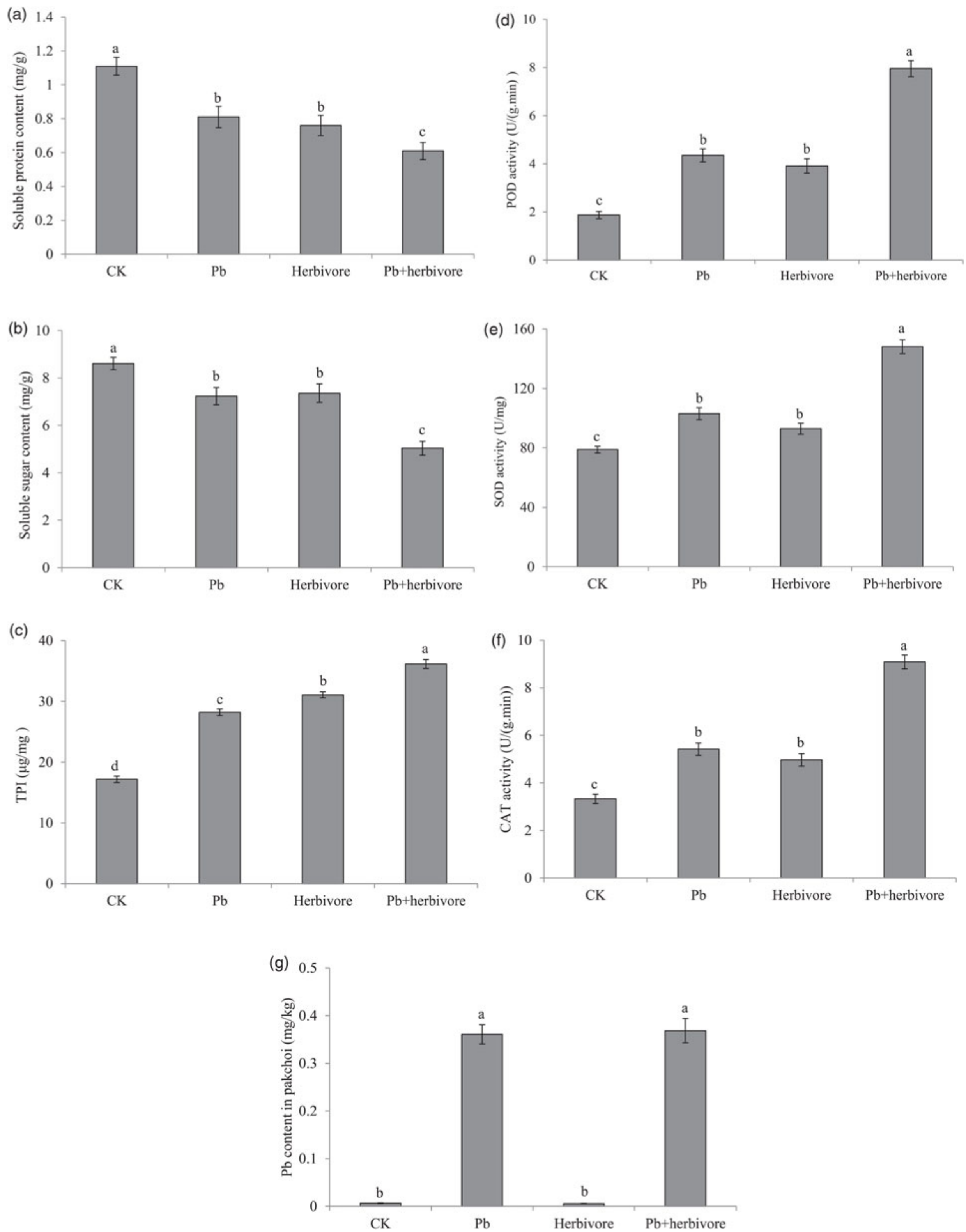

Figure 2. Leaf defensive chemicals and nutrient content in pakchoi plants under Pb stress, herbivore stress (*Spodoptera litura*), Pb and herbivore double stress and the control (CK). (a) Soluble protein content, (b) soluble sugar content, (c) trypsin protease inhibitor (TPI), (d) peroxidase (POD) activity, (e) superoxide dismutase (SOD) activity, (f) catalase (CAT) activity and (g) Pb content in pakchoi plants. Data are mean values ± standard error, and different lowercase letters indicate statistically significant differences as determined using Tukey's honestly significant difference.

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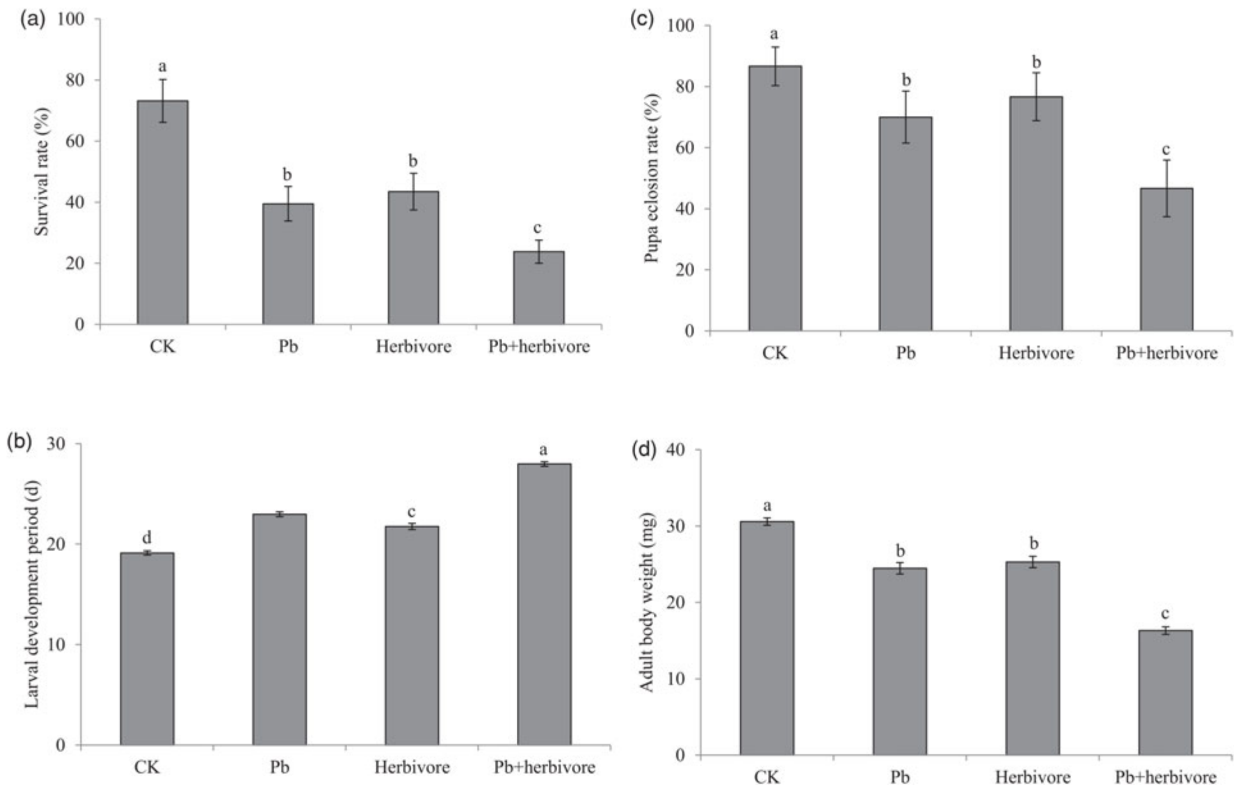

Figure 3. (a) Survival rate, (b) larval development period, (c) pupa eclosion rate and (d) adult body weight of *Spodoptera litura* larvae fed with pakchoi and under Pb stress, herbivore stress, Pb and herbivore double stress and the control (CK). Data are mean values ± standard error, and different lowercase letters indicate statistically significant differences as determined using Tukey's honestly significant difference.

the controls, the emergence rate decreased by 16.67, 10 and 40% in the Pb, herbivore and double stress groups, respectively. There was no significant difference between the Pb and herbivore treatment groups ($P > 0.05$) (fig. 3c).

The adult dry weight was significantly affected by Pb stress ($F_{1, 80} = 123.02$, $P < 0.05$), herbivore treatment ($F_{1, 80} = 82.11$, $P < 0.05$) and their combined effects ($F_{1, 80} = 4.65$, $P < 0.05$). Compared with that in the controls, the adult dry weight decreased by 6.1, 5.27 and 14.26 mg in the Pb, herbivore and double stress groups, respectively. There was no significant difference between the Pb and herbivore treatment groups ($P > 0.05$) (fig. 3d).

Discussion

This study showed that Pb, *S. litura* and their combined effects significantly affected soil enzymes, pakchoi and *S. litura*. Compared with the control groups, the following was observed: soil urease, phosphatase and sucrase activities decreased; the soluble protein and soluble sugar content of pakchoi plants decreased; the TPI content and POD, SOD and CAT activities increased; and *S. litura* growth and development were inhibited. Pb adversely affected soil–plant–herbivore interactions from bottom to top. It was shown that *S. litura* feeding affected plant–soil interactions from top to down and induced plant resistance. The combined effects of Pb and *S. litura* increased the plant defence response.

Soil enzyme activity is an important index that reflects soil characteristics (Long *et al.*, 2022). Soil urease activity can directly reflect the soil nitrogen supply, and phosphatase activity affects soil organophosphorus decomposition and conversion (Huang *et al.*, 2013; Long *et al.*, 2022). Sucrase activity can promote sugar hydrolysis and accelerate the soil carbon cycle (Jin *et al.*, 2009; Adetunji *et al.*, 2017). Liang *et al.* (2018) found that high Pb concentrations inhibited soil urease and phosphatase activities. Herbivory can directly or indirectly affect the composition and functioning of soil communities via changes in plant nutrients or defence compounds. For example, Classen *et al.* (2006) found that damage to *Pinus edulis* caused by *Matsucoccus acalyptus* significantly reduced soil enzyme activities. These findings that Pb or *S. litura* stress reduced soil enzyme activities agree with the results of this study. The present study also explored the influence of the combined effects of Pb and *S. litura* on enzyme activity. The results showed that soil enzyme activities were lower under double stress than under single stress, probably because the combined stress of Pb and *S. litura* exacerbated the changes in the soil environment and adversely affected enzyme activities related to the soil carbon, nitrogen and phosphorus cycles.

Herbivores feeding on plants produce herbivore-induced compounds that decrease the nutritional content of the plant, and these changes are detrimental to the growth and development of herbivorous insects and may lead to their death (Lou and Baldwin, 2003; Sznajder and Harvey, 2003; Li, 2016; Li *et al.*, 2021). Yin *et al.* (2012) found that the protein and glucose contents decreased in mustard, cabbage and kale after damage by *Plutella xylostella*. Moreover, the TPI content in soybean leaves increased following *S. litura* and *Spodoptera exigua* larval feeding; this was detrimental to the subsequent *S. litura* (Li, 2016). Dowd and Lagrimini (2006) found that *Manduca sexta* and *Heliothis virescens* larvae fed tobacco with a high POD content exhibited delayed growth and the associated harm to the plant was reduced.

The present study showed that *S. litura* stress reduced the nutrient content of pakchoi leaves, increased defence levels and inhibited *S. litura* growth and development.

Soil-mediated plant quality changes can alter aboveground plant–insect interactions (Kos *et al.*, 2015). Heavy metals in soil are absorbed by plant roots and transported to different tissues through various transporter proteins (Maksymiec *et al.*, 2005; Lei *et al.*, 2020). Excessively high heavy metal accumulation inhibits plant growth and development (Maksymiec *et al.*, 2005; Lei *et al.*, 2020). Mao *et al.* (2018) found that the protein content of soybean leaves decreased with increasing mercury (Hg) concentrations; however, SOD, POD and CAT activities first increased and then decreased. Antioxidant enzyme activity is inhibited when heavy metal concentrations exceed the plant's capacity. Heavy metals enter directly into the food chain and are toxic to insects; however, they also enhance the defence response of plants (Winter *et al.*, 2012). Cd-treated plants were more resistant to herbivorous insects than Cd-treated artificial feeds, indicating that Cd accumulation may activate plant defence responses (Li *et al.*, 2018). The present study showed that Pb stress reduced the soluble protein and soluble sugar contents and increased the defence levels of pakchoi plants. Pb inhibited *S. litura* growth and development, which may have resulted from synergistic effects of the elemental and chemical defences. In the Pb and *S. litura* double stress groups, Pb accumulation induced plant chemical defence, and pakchoi resistance induced by *S. litura* produced a superimposed or synergistic effect, resulting in an overall increase in the total defence level of pakchoi plants. In addition, the level of plant chemical defence is regulated under environmental stress by the allocation of plant resources (Boyd, 2012; Cheruiyot *et al.*, 2015; Sahu *et al.*, 2018; Chen *et al.*, 2020; Jiang *et al.*, 2020). This system can be considered in the context of the optimal defence theory (Barto and Cipollini, 2005; Kooyers *et al.*, 2017). Pakchoi plants may incur a level of pest damage, such that the produced chemical defence compounds exceed the growth and development of the plant. Thus, the plant utilises significant resources to synthesise defence-related compounds, thereby enhancing its chemical defence ability (Karban and Baldwin, 1997; Zavala *et al.*, 2004; Li, 2016).

In conclusion, the bottom–up effects of Pb on soil–pakchoi–*S. litura* interactions were analysed and the results showed that the simultaneous presence of elemental and chemical defences enhanced the insect resistance of pakchoi plants. In addition, the top–down effects of *S. litura* larval feeding on pakchoi, soil enzyme activity and subsequent *S. litura* infestation were explored. The results of this study provide novel information about the joint effects of Pb and herbivores on soil–plant–herbivorous insect interactions and management of crop pest control under heavy metal pollution. However, short-term toxicological tests on Pb were conducted here, and the cumulative effects and long–term nature of these effects were not considered. Further studies are, therefore, necessary to determine whether the effects of heavy metals can be extended to the progeny of herbivorous insects and their natural enemies at higher trophic levels.

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Author contributions. X. L. and X. Y. designed the study; H. L., Y. S. and Y. Z. performed the experiments; Z. S. and H. T. analysed the results and produced the figures; and X. L. wrote the manuscript. All authors have discussed and approved the final manuscript draft.

Competing interests. None.

References

Abdu N, Abdullahi AA and Abdulkadir A (2017) Heavy metals and soil microbes. *Environmental Chemistry Letters* **15**, 65–84.

Adetunji AT, Lewu FB, Mulidzi R and Ncube B (2017) The biological activities of *β*-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition* **17**, 794–807.

Aponte H, Meli P, Butler B, Paolini J, Matus F, Merino C, Cornejo P and Kuzyakov Y (2020) Meta-analysis of heavy metal effects on soil enzyme activities. *Science of the Total Environment* **737**, 139744.

Barto EK and Cipollini D (2005) Testing the optimal defense theory and the growth-differentiation balance hypothesis in *Arabidopsis thaliana*. *Oecologia* **146**, 169–178.

Bhaduri AM and Fulekar MH (2012) Antioxidant enzyme responses of plants to heavy metal stress. *Reviews in Environmental Science and Biotechnology* **11**, 55–69.

Bhattacharyya A, Leighton SM and Babu CR (2007) Bioinsecticidal activity of *Archidendron ellipticum* trypsin inhibitor on growth and serine digestive enzymes during larval development of *Spodoptera litura*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **145**, 669–677.

Boyd RS (2012) Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. *Plant Science* **195**, 88–95.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Butler CD and Trumble JT (2008) Effects of pollutants on bottom-up and top-down processes in insect-plant interactions. *Environmental Pollution* **156**, 1–10.

Cantarel AA, Pommier T, Desclos-Theveniau M, Diquélou S, Dumont M, Grassein F, Kastl EM, Grigulis K, Laîné P, Lavorel S, Lemauviel-Lavenant S, Personeni E, Schloter M and Poly F (2015) Using plant traits to explain plant-microbe relationships involved in nitrogen acquisition. *Ecology* **96**, 788–799.

Carrillo J, Ingwell LL, Li X and Kaplan I (2019) Domesticated tomatoes are more vulnerable to negative plant-soil feedbacks than their wild relatives. *Journal of Ecology* **107**, 1753–1766.

Chance B and Maehly AC (1955) Assay of catalases and peroxidases. *Methods in Enzymology* **2**, 764–775.

Chen J, Wang JW and Shu YH (2020) Review on the effects of heavy metal pollution on herbivorous insects. *Journal of Applied Ecology* **31**, 1773–1782.

Cheruiyot DJ, Boyd RS and Moar W (2015) Testing the joint effects hypothesis of elemental defense using *Spodoptera exigua*. *Journal of Chemical Ecology* **41**, 168–177.

Classen AT, DeMarco J, Hart SC, Whitham TG, Cobb NS and Koch GW (2006) Impacts of herbivorous insects on decomposer communities during the early stages of primary succession in a semi-arid woodland. *Soil Biology and Biochemistry* **38**, 972–982.

Dicke M (2015) Herbivore-induced plant volatiles as a rich source of information for arthropod predators: fundamental and applied aspects. *Journal of the Indian Institute of Science* **95**, 35–42.

Dowd PF and Lagrimini LM (2006) Examination of the biological effects of high anionic peroxidase production in tobacco plants grown under field conditions. I. Insect pest damage. *Transgenic Research* **15**, 197–204.

Gao Y, Zhou P, Mao L, Zhi YE and Shi WJ (2010) Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose–response model and PCR-RAPD. *Environmental Earth Sciences* **60**, 603–612.

Guan SY (1986) *Soil Enzyme and Research Methods*. Beijing, Agriculture Press, pp. 260–346.

Gupta AS, Webb RP, Holaday AS and Allen RD (1993) Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants). *Plant Physiology* **103**, 1067–1073.

Han P, Lavoir AV, Rodriguez-Saona C and Desneux N (2022) Bottom-up forces in agroecosystems and their potential impact on arthropod pest management. *Annual Review of Entomology* **67**, 239–259.

Hoysted GA, Bell CA, Lilley CJ and Urwin PE (2018) Aphid colonization affects potato root exudate composition and the hatching of a soil-borne pathogen. *Frontiers in Plant Science* **9**, 1278.

Hu LL (2004) *Intra- and Interspecific Interactions of Myzus Persicae (Sulzer) and Lipaphis Erysimi (Kaltenbach) on Cabbage (Brassica Oleracea var. Capitata)*. Shandong: Shandong Agricultural University.

Huang JH, Liu MQ, Chen XY, Chen J, Chen FJ, Li HX and Hu F (2013) Intermediate herbivory intensity of an aboveground pest promotes soil labile resources and microbial biomass via modifying rice growth. *Plant and Soil* **367**, 437–447.

Huang J, Chen Y, Huang Y and Yu J (2021) The effects of heavy metal accumulation on plant and herbivore interaction: a review. *Journal of Agriculture* **11**, 42–45.

Huberty M, Choi YH, Heinen R and Bezemer TM (2020) Above-ground plant metabolomic responses to plant-soil feedbacks and herbivory. *Journal of Ecology* **108**, 1703–1712.

Huberty M, Steinauer K, Heinen R, Jongen R, Hannula SE, Choi YH and Bezemer TM (2022) Temporal changes in plant-soil feedback effects on microbial networks, leaf metabolomics, and plant-insect interactions. *Journal of Ecology* **110**, 1328–1343.

Jiang D, Wang YY, Dong XW and Yan SC (2018) Inducible defense responses in *Populus alba berolinensis* to Pb stress. *South African Journal of Botany* **119**, 295–300.

Jiang D, Wang GR and Yan SC (2020) The improved resistance against gypsy moth in *Larix olgensis* seedlings exposed to Cd stress association with elemental and chemical defenses. *Pest Management Science* **76**, 1713–1721.

Jiang D, Tan M, Guo Q and Yan S (2021) Transfer of heavy metal along the food chain: a mini-review on insect susceptibility to entomopathogenic microorganisms under heavy metal stress. *Pest Management Science* **77**, 1115–1120.

Jin K, Sleutel S, Buchan D, De Neve S, Cai DX, Gabriels D and Jin JY (2009) Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau. *Soil and Tillage Research* **104**, 115–120.

Karban R (2011) The ecology and evolution of induced resistance against herbivores. *Functional Ecology* **25**, 339–347.

Karban R and Baldwin IT (1997) *Induced Responses to Herbivory*. Chicago, University of Chicago Press, pp. 33–38.

Kooyers NJ, Blackman BK and Holeski LM (2017) Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis. *Ecology* **98**, 1036–1048.

Kos M, Tuijl MA, de Roo J, Mulder PP and Bezemer TM (2015) Plant-soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilization. *Oikos* **124**, 658–667.

Lei GJ, Sun L, Sun Y, Zhu XF, Li GX and Zheng SJ (2020) Jasmonic acid alleviates cadmium toxicity in Arabidopsis via suppression of cadmium uptake and translocation. *Journal of Integrative Plant Biology* **62**, 218–227.

Li XH (2016) *Effects of Interaction Between Constitutive and Induced Resistance in Soybean Plants on Fitness Traits and Behavior of Spodoptera litura and its Parasitoid*. Najing: Najing Agricultural University.

Li J, Fang L, Lv Z and Zhang Z (2008) Relationships between the cotton aphid (*Aphis gossypii*) and the content of soluble sugars. *Plant Protection* **2**, 26–30.

Li K, Chen J, Jin P, Li J, Wang J and Shu Y (2018) Effects of Cd accumulation on cutworm (*Spodoptera litura*) larvae via Cd-treated Chinese flowering cabbage (*Brassica campestris*) and artificial diets. *Chemosphere* **200**, 151–163.

Li XH, Yang XJ, Huang ZY and Wu SL (2021) Effects of herbivore-feeding induced tobacco defenses on developmental performances and oviposition preference of *Spodoptera litura*. *Acta Tabacaria Sinica* **27**, 101–107.

Liang SX, Ding L, Shen S, Liu W, Li J and Xi X (2018) Assessment of the remediation effect of nano-hydroxyapatite in exogenous Pb-contaminated soil using toxicity characteristic leaching procedure and soil enzyme activities. *Bulletin of Environmental Contamination and Toxicology* **101**, 250–256.

Lin H, Wang Z, Liu C and Dong Y (2022a) Technologies for removing heavy metal from contaminated soils on farmland: a review. *Chemosphere* **305**, 135457.

Lin T, Zhu G, He W, Xie J, Li S, Han S, Li S, Yang C, Liu Y and Zhu T (2022b) Soil cadmium stress reduced host plant odor selection and oviposition preference of leaf herbivores through the changes in leaf volatile emissions. *Science of the Total Environment* **814**, 152728.

Long Y, Liu JP and Li XH (2022) Effects of *Spodoptera litura* feeding on soybean agronomic characters and soil enzyme activities. *Plant Protection* **48**, 122–128.

Lou YG and Baldwin IT (2003) *Manduca sexta* recognition and resistance among allopolyploid *Nicotiana* host plants. *Proceedings of the National Academy of Sciences* **100**(Suppl_2), 14581–14586.

Maksymiec W, Wianowska D, Dawidowicz AL, Radkiewicz S, Mardarowicz M and Krupa Z (2005) The level of jasmonic acid in *Arabidopsis thaliana* and *Phaseolus coccineus* plants under heavy metal stress. *Journal of Plant Physiology* **162**, 1338–1346.

Mao F, Nan G, Cao M, Gao Y, Guo L, Meng X and Yang G (2018) The metal distribution and the change of physiological and biochemical process in soybean and mung bean plants under heavy metal stress. *International Journal of Phytoremediation* **20**, 1113–1120.

Martijn Bezemer T, van der Putten WH, Martens H, van de Voorde TF, Mulder PP and Kostenko O (2013) Above–and below–ground herbivory effects on below–ground plant–fungus interactions and plant–soil feedback responses. *Journal of Ecology* **101**, 325–333.

Nurzhan A, Tian H, Nuralykyzy B and He W (2022) Soil enzyme activities and enzyme activity indices in long-term arsenic-contaminated soils. *Eurasian Soil Science* **55**, 1425–1435.

Orwin KH, Buckland SM, Johnson D, Turner BL, Smart S, Oakley S and Bardgett RD (2010) Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of Ecology* **98**, 1074–1083.

Paudel S, Lin PA, Foolad MR, Ali JG, Rajotte EG and Felton GW (2019) Induced plant defenses against herbivory in cultivated and wild tomato. *Journal of Chemical Ecology* **45**, 693–707.

Pineda A, Zheng SJ, van Loon JJ, Pieterse CM and Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends in Plant Science* **15**, 507–514.

R Development Core Team (2022) *R Language*. R Foundation Statistical Computing. Available at https://www.R-project.org/

Sahu S, Dutta A, Ray DK, Pradhan J and Dandapat J (2018) Host plant-derived allelochemicals and metal components are associated with oxidative predominance and antioxidant plasticity in the larval tissues of silkworm, *Antheraea mylitta*: further evidence of joint effects hypothesis. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **223**, 39–49.

Sang W, Xu J, Bashir MH and Ali S (2018) Developmental responses of *Cryptolaemus montrouzieri* to heavy metals transferred across multi-trophic food chain. *Chemosphere* **205**, 690–697.

Sidhu GPS, Bali AS, Bhardwaj R, Singh HP, Batish DR and Kohli RK (2018) Bioaccumulation and physiological responses to lead (Pb) in *Chenopodium murale* L. *Ecotoxicology and Environmental Safety* **151**, 83–90.

Song GC, Lee S, Hong J, Choi HK, Hong GH, Bae DW, Mysore KS, Park YS and Ryu CM (2015) Aboveground insect infestation attenuates below-ground *Agrobacterium*-mediated genetic transformation. *New Phytologist* **207**, 148–158.

Sznajder B and Harvey JA (2003) Second and third trophic level effects of differences in plant species reflect dietary specialization of herbivores and their endoparasitoids. *Entomologia Experimentalis et Applicata* **109**, 73–82.

Tan XP, He JH, Guo ZM, Wang ZQ, Nie YX, Ye Q, He WX and Shen WJ (2023) Research progresses on soil enzymes as indicators of soil health and their responses to heavy metal pollution. *Acta Pedologica Sinica* **60**, 50–52.

Tang B, Xu H, Song F, Ge H and Yue S (2022) Effects of heavy metals on microorganisms and enzymes in soils of lead-zinc tailing ponds. *Environmental Research* **207**, 112174.

Tibbett M, Green I, Rate A, De Oliveira VH and Whitaker J (2021) The transfer of trace metals in the soil-plant-arthropod system. *Science of the Total Environment* **779**, 146260.

Wang M, Ruan W, Kostenko O, Carvalho S, Hannula SE, Mulder PP, Bu F, van der Putten WH and Bezemer TM (2019) Removal of soil biota alters

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soil feedback effects on plant growth and defense chemistry. *New Phytologist* **221**, 1478–1491.

Watanabe T and Kitagawa H (2000) Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *Journal of Economic Entomology* **93**, 1192–1198.

Winiarska-Mieczan A, Jachimowicz K, Kwiecień M, Krusiński R, Kislova S, Sowińska L, Zasadna Z and Yanovych D (2023) The content of Cd and Pb in herbs and single-component spices used in Polish cuisine. *Biological Trace Element Research* **201**, 3567–3581.

Winter TR, Borkowski L, Zeier J and Rostás M (2012) Heavy metal stress can prime for herbivore-induced plant volatile emission. *Plant, Cell and Environment* **35**, 1287–1298.

Yactayo-Chang JP, Tang HV, Mendoza J, Christensen SA and Block AK (2020) Plant defense chemicals against insect pests. *Agronomy* **10**, 1156.

Yin F, Feng X, Zhang DY, Li ZY, Lin QS, Hu ZD and Chen HY (2012) Contents changes of protein and sugar in host plants after damaged by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Journal of Environmental Entomology* **34**, 168–173.

Zavala JA, Patankar AG, Gase K and Baldwin IT (2004) Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. *Proceedings of the National Academy of Sciences of the USA* **101**, 1607–1612.

Zhang SW, Zong YJ, Fang CY, Huang SH, Li J, Xu JH, Wang YF and Liu CH (2020) Optimization of anthrone colorimetric method for rapid determination of soluble sugar in barley. *Food Research and Development* **41**, 196–200.

Zhang Q, Zhou M and Wang J (2022) Increasing the activities of protective enzymes is an important strategy to improve resistance in cucumber to powdery mildew disease and melon aphid under different infection/infestation patterns. *Frontiers in Plant Science* **13**, 950538.

Zhao A, Li Y, Leng C, Wang P and Li Y (2019) Inhibitory effect of protease inhibitors on larval midgut protease activities and the performance of *Plutella xylostella* (Lepidoptera: Plutellidae). *Frontiers in Physiology* **9**, 1963.

https://doi.org/10.1017/S0007485324000208 Published online by Cambridge University Press