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RESEARCH ARTICLE

Trophic transfer and bioaccumulation of lead along soil–plant–aphid–ladybird food chain

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Abstract

Lead (Pb) contamination of agroecosystems is a serious issue as Pb is a persistent pollutant that is retained in soil for long, causing toxicities to organisms. This study examines biotransfer of Pb from soils treated with different concentrations of Pb through a broad bean (*Vicia faba* L.)–aphid (*Aphis fabae* Scop.)–ladybird (*Coccinella transversalis* Fabricius) food chain and its consequent inference for natural biological control, the ladybird. The soil was amended with Pb at the rates of 0, 25, 50, 75 and 100 mg kg^{-1} (*w*/*w*). The amount of Pb in plant, aphid and ladybird increased in a dose-dependent manner to Pb contents in the soil. The results showed that Pb biomagnified from soil to root with transfer coefficient always > 1. Biominimization of Pb occurred at the second trophic level in aphids and at the third trophic level in ladybirds as their respective transfer coefficients from shoot to aphid and aphid to ladybird were always < 1. The increased elimination of Pb via aphid excreta (honeydew) and pupal exuviae in a dose-dependent manner suggests that these are possible detoxification mechanisms at two different trophic levels which control Pb bioaccumulation along the food chain. The statistically significant ($p \leq 0.05$) decreases in biomass and predation rate of predatory ladybirds at 100 mg kg^{-1} Pb indicate that high dose of Pb in soil may have sub-lethal effects on ladybirds. Further studies at cellular and sub-cellular levels are needed to further document the potential mechanisms of achieving Pb homeostasis in ladybirds under Pb stress.

Keywords Accumulation · Lead · Trophic level · Aphid · Ladybird · Biomagnification

Introduction

Agroecosystem contamination by lead (Pb), a toxic heavy metal, due to intensified anthropogenic activities, including industrial operations, mining, smelting, as well as use of sewage sludge, fly ash and Pb-containing pesticides and fertilizers in agriculture, has become a serious issue of global concern (Dar et al. 2015, 2017; Anjum et al. 2016). These activities may release Pb into the environment via waste disposal and runoff and through the application of Pb-containing chemical products which enter the ecosystem through soil, aerial deposition and surface waters (Boyd 2004; Chaffai and Koyama 2011; Gall et al. 2015). Lead is a persistent pollutant and may retain in the soil even after the point source is removed (Babin-Fenske and Anand 2011). Lead is a toxic element with serious health effects on humans and other biota. Plants serve as a route of exposure of Pb to herbivores while herbivores serve as a source of Pb transfer to organisms at higher levels in the food chain (Devkota and Schmidt 2000; Zhang et al. 2017). Aphids are harmful pests and can severely damage crop plants (Rehman et al. 2014). Insect predators play a vital ecological role in pest management, thereby minimizing economic losses in pest-ridden crops (Winder et al. 1999; Green et al. 2003). The natural biocontrol of pests by insect predators is of great economic importance due to high price of pesticides (Dar et al. 2015) as well as their harmful effects on plants (Dar et al. 2016). The consumption of Pb contaminated prey may have negative effects on the wellbeing of predatory insects, thereby reducing effectiveness of pest management (Merrington et al. 2001). Thus, the safe disposal of heavy metal containing

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wastes in agricultural settings is vital for safeguarding natural biological control agents.

In our previous studies, heavy metals (Cd, Pb and Zn) from sewage sludge and fly ash-amended soil showed differences in accumulation along the food chain in relation to whether heavy metal transfer was elevated or limited (Dar et al. 2015, 2017). Lead showed retention in ladybird tissues during pupation with increase in Pb exposure as sewage sludge treatments increased (Dar et al. 2015) while its biominimization was observed with the addition of fly ash (Dar et al. 2017). There are currently gaps in the research showing the effects of different sources of potentially toxic metals on trophic transfer in multi-trophic food chains.

In this sense, as indicated by previous studies, fly ash usually has a low content of organic matter (as it is almost completely eliminated in the combustion process) and is alkaline whereas sewage sludge contains a large amount of organic compounds and is slightly acidic. Fly ash amendment has shown contrasting results: Tsadilas et al. (2009) and Shaheen and Tsadilas (2010) observed increased soil metal sorption and reduced uptake of trace metals by plants, while Gupta and Sinha (2009) witnessed increased Pb translocation from root to shoot as well as an increase in its concentration in *Vigna radiata* (mung bean) shoots. Therefore, a study using direct Pb contamination by spiking Pb in the soil in the form of Pb $(NO_3)_2$ may provide an interesting comparison with previous studies carried out using fly ash and sewage sludge as Pb source. Lead mobility in the third trophic level observed by Dar et al. (2017) is an indication that Pb contamination may be more problematic than has been realised (Dar et al. 2017). Lead is usually restricted mainly to root–shoot transfer in the food chain (Dar et al. 2015), but Pb spiking of soil may enhance biotransfer of Pb in the higher trophic levels of the food chain.

The present study is aimed at investigating the magnitude of biotransfer of Pb from soil spiked with different concentrations of Pb through a plant (*Vicia faba* L.)–aphid (*Aphis fabae* Scop.)–predatory ladybird (*Coccinella transversalis* Fabricius) food chain and its subsequent impact on the natural biological control agent, the ladybird. *Vicia faba* (broad/faba bean) was selected as the primary trophic level because of its large biomass and widespread geographic cultivation. *Aphis fabae* (the black bean aphid) is a harmful insect pest of broad bean and forms the second trophic level of the food chain and *Coccinella transversalis* (ladybird), the adults and larvae of which are voracious feeders of aphids, were selected as predators at the third trophic level.

Materials and methods

The sandy loam soil used in the present study was collected from Aligarh Muslim University (AMU) agriculture farm fields, air dried, filled in pots and treated with Pb stock solution at concentrations of 0, 25, 50, 75 and 100 mg kg^{-1} of Pb and designated as T0, T1, T2, T3, and T4, respectively. Lead in the form of lead nitrate $(Pb(NO_3)_2)$ was dissolved in double distilled water (DDW) to make Pb stock solution of 10 g/l which was then used to spike the soil as described above. All treatments were replicated four times. The soil-filled pots (5.0 kg in each pot) were moistened to field capacity and incubated for 3 weeks to equilibrate as described by Dar et al. (2015). After the incubation period, soils were sampled from each pot for examining soil properties, including total and bioavailable Pb concentrations.

After incubation of soil, five seeds of broad bean were sown in each pot at a depth of 0.5 cm. The pots placed in a fully randomised block design were kept in a glasshouse at AMU (set to 26 °C ± 1 °C and 16:8 h day–night regime) and were watered regularly with DDW. Plastic plates were placed below each pot to collect the percolated water and leachate, which were reused when watering. At five to six leaf stages of seedlings, thinning was done and three seedlings of equal growth and vigour were retained in each pot.

To study the effects of Pb on the growth of broad bean, one plant from each pot was uprooted at 40 days after sowing (DAS) for the analysis of chlorophyll content and dry mass. At the flowering stage, 200 broad bean aphids (*Aphis fabae*) cultured in the laboratory were transferred to the broad bean plants growing in each pot. Individual pots were subsequently covered with sleeve cages to prevent the movement of aphids between treatments (Dar et al. 2015). After 3 weeks of infestation, aphids were collected from each pot following Dar et al. (2015). The collected aphid samples were divided into two groups, one from each treatment for quantification of Pb and another kept at − 18 °C in a freezer (DW-40L626, Haier Biomedical, China) for feeding predatory ladybirds.

Broad bean plants were also sampled for Pb content at this time. Pb concentration was also determined in the honeydew of aphids following Crawford et al. (1995). The feeding experiment of predatory ladybirds was done following Dar et al. (2015). Twenty larvae (4th instar) of predatory ladybirds were divided into five equal treatment groups. Each individual larva, reared separately in a petri dish of 9 cm diameter with moistened filter paper, was fed with frozen aphids collected from the pot cultures. Each petri dish was covered by fine nettings secured with a rubber band and kept in a controlled environment cabinet (25 °C and 16:8 h day–night regime). Aphid consumption by individual larva was observed every 24 h and fresh aphids were added to petri dishes as needed. The larvae of ladybirds were fed until pupation. At pupation, newly emerged adult ladybirds were weighed and frozen at − 18 °C for the analysis of Pb.

Analysis of soil properties

The soil pH for the different treatments was measured in a soil:water suspension (1:2.5 *w*/*v*) with a digital pH meter (M-181, India). Soil organic carbon content was determined by Walkley and Black's rapid titration method (Jackson 1958). Total nitrogen content in the soil samples was determined by the Micro-Kjeldahl method (Bremner 1960).

Lead analysis

Soil samples collected in triplicate from each pot were air dried and crushed before passing through a fine sieve (2-mm mesh). The soil sample equal to 1 g was digested in 20 ml of concentrated tri-acid mixture ($HNO_3/H_2SO_4/HClO_4$, 5:1:1 *v*/*v*) at 80 °C (Allen et al. 1986). After digestion, the solution was cooled and then filtered through a Whatman no. 42 filter paper. The filtered solution was diluted to 50 ml with DDW prior to analysis for total Pb concentration by atomic absorption spectrometry (AAS) (GBC, 932 plus; GBC Scientific Instruments, Braeside, Australia). The AAS was operated with an air/acetylene flame and was equipped with a Pb hollow cathode lamp working at 5.0 mA current, 217.0 nm wavelength and 1.0 slit width. The instrument was calibrated by using Milli-Q water as blank and three solutions of known Pb concentrations, prepared by dilution of a Pb standard stock solution (1000 µg ml^{-1}). A linear calibration curve (absorbance vs concentration) was obtained and a detection limit of 0.1 ppm was calculated for Pb. The bioavailable fraction of Pb in soil was determined by extraction with 0.005 M diethylene triamine pentaacetic acid (DTPA) buffered with triethanolamine to pH 7.3 (Lindsay and Norvell 1978) for 2 h and subsequent detection by AAS.

Broad bean plants harvested from each pot were washed with tap water to remove the soil and rewashed with DDW. Roots and shoots were separated, dried in an oven at 70 °C for 72 h and then finely powdered and sieved, using a kitchen electronic grinder and a 2-mm mesh sieve, respectively. Plant samples equal to 0.3 g were digested in 10 ml of concentrated tri-acid mixture (HNO_3/ H_2SO_4/ $HClO_4$; 5:1:1 *v*/*v*) at 80 °C (Allen et al. 1986). The digested solution was allowed to cool and diluted with DDW and subsequently filtered through a Whatman no. 42 filter paper. The filtrate was then diluted to 50 ml with DDW. The sub-samples of dried aphids, predatory ladybirds and their exuviae (from each treatment) were digested by following the method of Dar et al. (2015). Aphid sub-samples and ladybirds were washed with DDW and then dried as described for plant samples. Individual ladybirds and 20-mg sub-samples of aphids were digested in 2.0 ml of concentrated tri-acid mixture ($HNO_3/H_2SO_4/HClO_4$, 5:1:1 *v*/*v*) at 80 °C. The digested solution was diluted to 5 ml using DDW. Pupal exuviae (0.5 mg) were also digested in a similar way, using a 2.0-ml acid mixture, then volume was made back to 2.0 ml with DDW. The total Pb concentrations in all samples were determined by AAS as described above. All chemicals and reagents used in the study were of analytical grade and supplied by Sigma-Aldrich. Analytical quality was checked by the analysis of certified reference materials, i.e., GBW 07402 for soil, NIM-GBW10048 (celery plant) for plants and GBW 08552 (pork muscle) for insects. Mean recoveries from these materials for Pb was 96.5%. Procedural blanks were also digested to check for process contamination.

Data analysis and calculation

Data were analysed using SPSS software (IBM SPSS Statistics 20) to determine the significance at $p < 0.05$. Duncan's multiple range test (DMRT) was performed to test the significance of differences among treatments. Transfer coefficients (TC) for Pb in the food chain were calculated by determining the ratio of concentration of a metal at the receiving level to the concentration of this metal at the source level. Lead concentrations in the pupa of ladybirds before adult emergence were determined by adding the metal content of the newly emerged adult to that of the exuviae. The percent of metal burden lost through pupal exuviae was calculated as:

$$\text{Percent of metal burden lost via exuviae} = \frac{\text{Metal contents in Individual beetle}}{\text{Metal contents in individual pupa}} \times 100$$

Results

Effect of lead treatment on soil properties

The soil used in the experiment had pH 7.84 ± 0.04 with organic carbon (%) 0.69 ± 0.02 and nitrogen content 0.17 ± 0.02 (Table 1). The soil pH only slightly decreased at Pb amendments of 75 and 100 mg kg^{-1} soil (T3 and T4) (Table 1). The soil amended with different concentrations of Pb had statistically ($p > 0.05$) similar soil organic matter content in all the treatments. The Pb amendments in the soil significantly ($p < 0.05$) altered the nitrogen content (Table 1). The nitrogen content of the soil was significantly ($p < 0.05$) higher in T4 and T3 compared with T0 (Control) whereas the total nitrogen content in T0, T1 and T2 were statistically ($p > 0.05$) similar. The total and DTPA extractable levels of soil Pb concentrations increased significantly ($p < 0.05$) in a dose-dependent manner of Pb treatments (Table 1).

Table 1 Chemical properties and Pb concentrations (total and extractable) of soil treated with different doses of Pb (mean ± SE, $n = 4$). Values with different small superscript letters in each group are significantly different from each other at $p < 0.05$

Parameters	T0	T1	T2	T3	T4
pH	7.84 ± 0.04^{a}	7.82 ± 0.03^{a}	7.81 ± 0.05^{ab}	7.78 ± 0.06^{b}	7.76 ± 0.03^{b}
Organic C (%)	0.69 ± 0.02^{a}	0.70 ± 0.02^{a}	0.70 ± 0.03^{a}	0.72 ± 0.02^{a}	0.73 ± 0.02^{a}
Total *N* (%)	0.17 ± 0.02^{c}	0.19 ± 0.02^{c}	0.21 ± 0.03^{b}	0.23 ± 0.04^{b}	0.27 ± 0.05^{a}
Total lead in soil (mg kg^{-1})					
Pb	10.86 ± 0.44^{e}	35.32 ± 2.07^{d}	58.78 ± 2.72^{c}	82.35 ± 3.71^{b}	105.29 ± 6.31^{a}
DTPA extractable lead (mg kg^{-1})					
Pb	1.96 ± 0.11^{e}	6.35 ± 0.42^{d}	9.84 ± 0.39^{c}	14.90 ± 0.82^{b}	19.09 ± 1.04^{a}

T0, control soil; *T1*, 25 mg kg^{-1}; *T2*, 50 mg kg^{-1}; *T3*, 75 mg kg^{-1}; *T4*, 100 mg kg^{-1} Pb treatments

Effects of Pb on the growth of broad bean

The chlorophyll content (both cholorophyll a and b) decreased significantly ($p < 0.05$) in a dose-dependent manner as compared with the control (Fig. 1 a, b). The plant biomass (root and shoot dry mass) decreased significantly ($p < 0.05$) upon the addition of varying Pb doses as compared with the control (Fig. 1c).

Pb transfer between soil and broad bean roots

The Pb concentration in roots increased significantly ($p < 0.05$) when grown in Pb contaminated soils. The maximum Pb concentration of 129.51 mg kg^{-1} dry roots was recorded for T4 treatment (Fig. 2a). Lead content in the roots of control (T0) was 10.82 mg kg^{-1} dry roots (Fig. 2a). Transfer coefficients of Pb between the soil and broad bean roots increased with an increase in Pb amendments and ranged from 1.00 to 1.23 for total Pb to root, and 5.52 to 6.78 for soil DTPA extractable fraction to root total concentration (Table 2).

The mean dry mass of roots decreased significantly ($p < 0.05$) with the increase in the doses of Pb as compared to the control. The highest root dry mass, 0.425 ± 0.05 g plant^{-1} was recorded for T0 whereas the lowest, 0.321 ± 0.04, was recorded for T4, showing a decline of 24.62% (Fig. 1c).

Pb transfer between roots and shoots

Lead content in the shoots of the broad bean differed significantly ($p < 0.05$) among different Pb treatments. The highest accumulation of Pb in the shoots of broad bean was 72.52 mg kg^{-1} dry shoots for T4 (Fig. 2b). Lead content accumulated in the shoots of control (T0) was 5.84 mg kg^{-1} dry shoots (Fig. 2b). Transfer coefficients of Pb between the broad bean roots and shoots were almost identical for all treatments and remained below 1 (around 0.54) in all the Pb amendments (Table 2).

The mean dry mass of shoots decreased significantly ($p < 0.05$) with the increase in the doses of Pb treatments. The highest shoot dry mass, 1.843 ± 0.09 g plant^{-1}, was recorded for T0 whereas the lowest, 1.417 ± 0.05, was recorded for T4, showing a decline of 23.12% (Fig. 1c).

Pb transfer between shoots and aphids

The lead content in the bodies of aphids fed on broad bean shoots contaminated with Pb increased significantly ($p < 0.05$) with the increase of Pb. The maximum accumulation of Pb (63.82 mg kg^{-1} dry weight) in the bodies of aphids was observed for T4 (Fig. 2c). Lead content in the aphids reared on control (T0) plants was recorded as 5.03 mg kg^{-1} dry weight (Fig. 2c). Transfer coefficients (TC) of Pb between broad bean shoots and aphids was higher than the TC of root to shoot, but remained below 1 (around 0.85) and was similar in all Pb amendments (Table 2).

Pb transfer between aphids and ladybirds

The lead content in newly emerged adult ladybirds increased significantly ($p < 0.05$) when fed on aphids nurtured on Pb-treated plants (Fig.2d). The maximum accumulation of Pb (42.75 mg kg^{-1} dry weight) in adult ladybirds was observed for T4 (Fig.2d). Lead content in ladybirds reared on aphids fed with control (T0) plants was recorded at 3.32 mg kg^{-1} dry weight (Fig. 2d). The transfer coefficient (TC) of Pb between aphids and ladybirds remained below 1 (~0.67), irrespective of the Pb treatment levels (Table 2).

Pb excretion with honeydew of aphids

Some of the Pb in the bodies of aphids was eliminated via honeydew and its concentration increased with increase in the Pb treatments (Table 3). The concentration of Pb in the honeydew of aphids nurtured on broad bean plants grown in control soil (T0) was 0.75 mg kg^{-1} dry weight whereas in T4, it was 16.59 mg kg^{-1} dry weight. The percentage (%) of Pb in

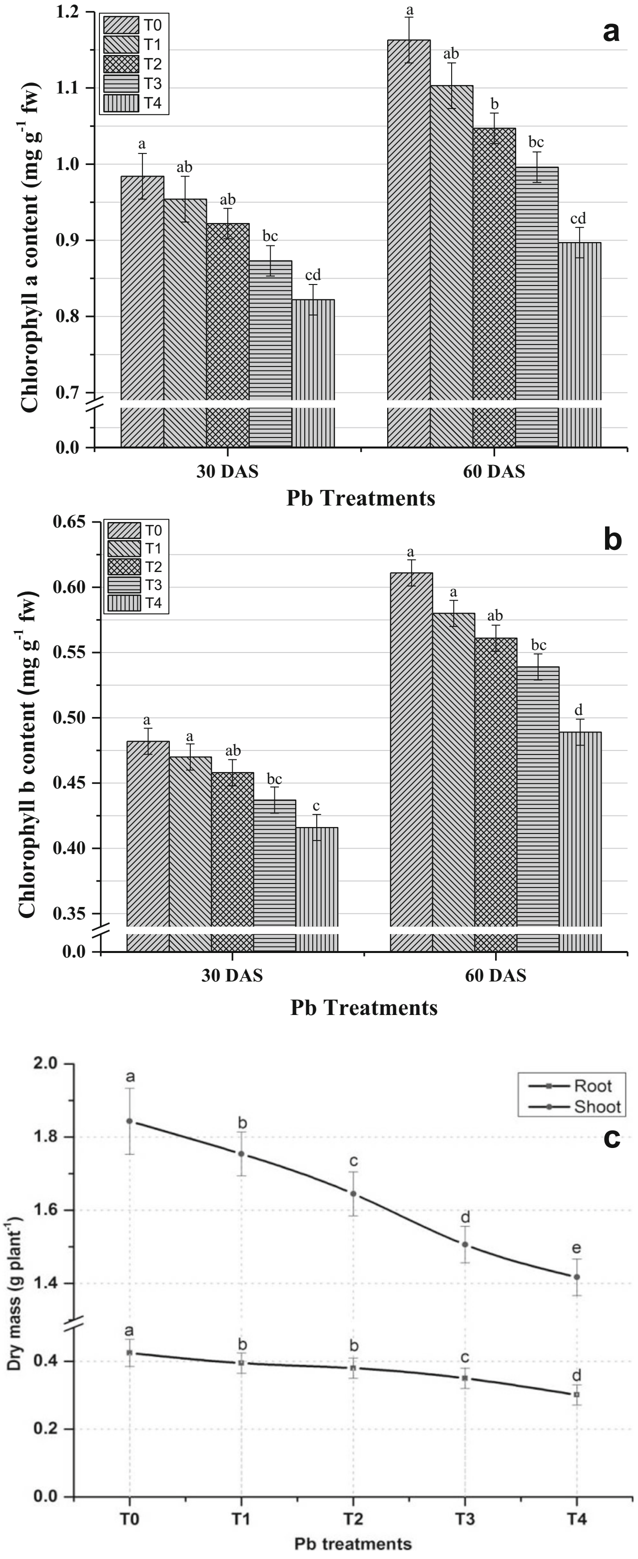

Fig. 1 Effect of various levels of Pb (T0, control soil; T1, 25 mg kg^{-1}; T2, 50 mg kg^{-1}; T3, 75 mg kg^{-1}; T4, 100 mg kg^{-1} Pb treatments) on chlorophyll a **(a)**, chlorophyll b **(b)** content of *Vicia faba* L. at 30 and 60 days after sowing (DAS) and dry mass of *Vicia faba* L. **(c)** (mean ± SE; $n = 4$). Different small letters at each growth stage show statistically significant variation at $p < 0.05$ as per DMRT

honeydew of aphids (Table 3, within parenthesis) increased with the increase in Pb concentrations and ranged from 15 to 26%.

Pb sequestered in pupal exuviae of ladybirds

Significant differences ($p < 0.05$) in the elimination of Pb via pupal exuviae of predatory ladybirds were found among the different Pb treatments (Table 3). The concentration of Pb in the pupal exuviae was 1.81 mg kg^{-1} dry weight in control (T0) and 37.05 mg kg^{-1} dry weight in T4. The percent (%) sequestration of metal burden in exuviae (Table 3, within parenthesis) increased with the increase in Pb concentrations.

Effects of Pb on predation rate of ladybirds

The predation rate (average consumption of aphid) by ladybirds was not significantly ($p < 0.05$) affected when fed on aphids raised on broad bean plants treated with lower doses of Pb (T0 to T3) but the predation rate decreased significantly ($p < 0.05$) when fed on aphids raised on broad bean plants grown under higher doses (T4) of Pb (Fig. 3).

Effects of Pb on biomass of aphids and ladybirds

The higher dose of Pb, 100 mg kg^{-1} soil, reduced the fresh and dry mass of aphids significantly ($p < 0.05$) as compared with the control (Table 4). Similarly, with the elevation in the Pb treatment levels, fresh and dry mass of newly emerged adult ladybirds decreased significantly ($p < 0.05$; Table 4).

Discussion

Effects of Pb spiking on soil properties

In the present study, soil spiked with different concentrations of Pb did not alter the total organic matter content, as expected and reported by Zhang et al. (2017) and Singh and Agarwal (2010). The increase in the total nitrogen content is due to the addition of Pb as a salt of nitrate ($Pb(NO_3)_2$). The total soil Pb and its phytoavailable (DTPA extractable) portion significantly increased on increasing Pb amendment, but remained below the Indian permissible limits (250–500 mg kg^{-1}) in all the Pb treatments (Awashthi 2000; Nicholson and Chambers 2008).

Effects of Pb on broad bean growth

Plant biomass and chlorophyll content of leaves are important measures to assess various environmental stresses on plants (Ashraf and Harris 2013; Dwivedi et al. 2007), as both are associated with plant growth, productivity and vigour. In the

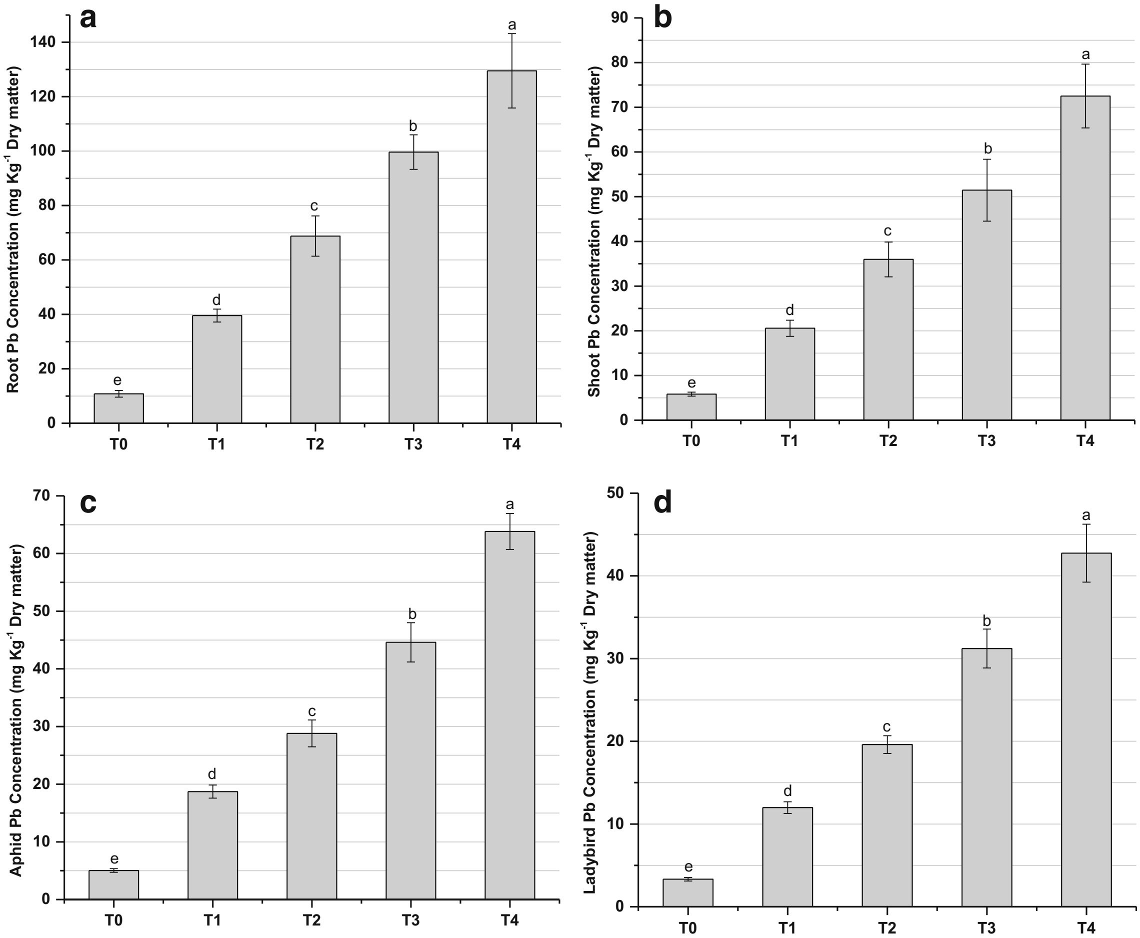

Fig. 2 Lead concentrations (mg kg^{-1} dry weight; mean ± SE; $n = 4$) in broad bean roots **(a)**, broad bean shoots **(b)**, aphids (*Aphis fabae*) **(c)** and predatory ladybirds (*Coccinella transversalis*) **(d)** treated with different doses of Pb (T0, control soil; T1, 25 mg kg^{-1}; T2, 50 mg kg^{-1}; T3, 75 mg kg^{-1}; T4, 100 mg kg^{-1} Pb treatments.). Bars with different small letters on the top are significantly different from each other at $p < 0.05$

present study, the photosynthetic pigments (chlorophyll-a, chlorophyll-b) decreased in 30 and 60 days in old broad bean plants grown in Pb-amended soil. These findings are further corroborated by the studies of Shahid et al. (2014) revealing the reduction in chlorophyll content in the leaves of *Vicia faba* under Pb stress. Additional studies have also revealed that Pb decreases the chlorophyll content in the leaves of ryegrass (Bai et al. 2015), cotton (Khan et al. 2016) and buckwheat (Hakeem et al. 2018). Lead-induced decrease in pigment content may be due to distorted chloroplast ultrastructure or the inhibition in the activity of δ-aminolevulinic acid dehydratase (ALAD) (John et al. 2008; Gupta et al. 2009), involved in the biosynthesis of photosynthetic pigments (Shu et al. 2012), thus leading to the reduction in pigment content (Hou et al. 2018). The Pb-induced generation of reactive oxygen species (ROS) is also believed to reduce chlorophyll content by interfering with photosynthetic apparatus and mechanisms involved in pigment synthesis (Shahid et al. 2012). The major adverse effects of Pb stress include damage to the oxygen-evolving complex (OEC), inhibition of photosystem (PS) II and I activity, disruption of electron transport between OEC and PSII-reaction centre (RC) and energy transfer to PSII-RC (Parys et al. 2014; Kumar and Prasad 2015; Rodriguez et al. 2015), and the inhibition of the Calvin Cycle (Pourrut et al. 2013).

Table 2 Transfer coefficients of Pb concentrations between various components of the soil–plant–aphid–ladybird food chain on amendment of soil with various levels of Pb

Food chain components	T0	T1	T2	T3	T4
Total Pb soil to root	1.00	1.12	1.17	1.21	1.23
Extractable Pb soil to root	5.52	6.22	6.99	6.69	6.78
Root to shoot	0.54	0.52	0.52	0.53	0.56
Shoot to aphid	0.86	0.91	0.80	0.87	0.88
Aphid to ladybird	0.66	0.64	0.68	0.70	0.67

T0, control soil; *T1*, 25 mg kg^{-1}; *T2*, 50 mg kg^{-1}; *T3*, 75 mg kg^{-1}; *T4*, 100 mg kg^{-1} Pb treatments

Plants growing in Pb-treated soils exhibit detrimental effects on the growth and biomass (Chen et al. 2016). The

Table 3 Pb content (mg kg^{-1} dry weight) excreted via honeydew of aphids and in pupal exuviae of predatory ladybirds exposed to varying levels of Pb concentration (mean ± SE; $n = 4$). Values with different small superscript letters in each group are significantly different from each other at $p < 0.05$

Treatments	Honeydew	Pupal exuviae
T0	0.75 ± 0.03^{e}	1.81 ± 0.24^{e}
	(15)	(7.12)
T1	3.37 ± 0.18^{d}	8.99 ± 0.96^{d}
	(18)	(9.66)
T2	5.76 ± 0.38^{c}	13.53 ± 1.25^{c}
	(20)	(9.07)
T3	10.26 ± 0.85^{b}	21.85 ± 1.55^{b}
	(23)	(9.10)
T4	16.59 ± 1.27^{a}	37.05 ± 2.78^{a}
	(26)	(11.70)

T0, control soil; *T1*, 25 mg kg^{-1}; *T2*, 50 mg kg^{-1}; *T3*, 75 mg kg^{-1}; *T4*, 100 mg kg^{-1} Pb treatments

Values within parenthesis are percentages of Pb content excreted via honeydew of aphids and in pupal exuviae of predatory ladybirds

decrease in the root and shoot dry mass in the present study are in close conformity with the findings of Wang et al. (2010) of Pb phytotoxicity in *Vicia faba* seedlings. The reduction in plant biomass may also be due to Pb-mediated disruption in water transport system in the plant tissues or inhibition of mitotic index and cell elongation (Kumar et al. 2012; Rucińska-Sobkowiak et al. 2013).

Pb transfer from soil to root

The roots of *Vicia faba* plants accumulated the highest concentration of Pb among the various components of the studied food chain, and it increased in a concentration dependent manner. The transfer coefficients of Pb from soil to roots were highest compared with other components of the food chain. Root Pb concentrations were higher than critical Pb limits (2–6 μg kg^{-1}) as described by Kabata-Pendias and Pendias (2011). These results parallel previous findings of Probst et al. (2009) who observed increased Pb concentrations in *Vicia faba* roots. Similarly, Dar et al. (2015, 2017), Alexander et al. (2006) and Wang et al. (2006) also reported that roots accumulated higher amounts of metals as compared with other parts of plants. Lead, being a non-essential element, does not have any distinct transport mechanisms for its uptake and its entry to the plant root cells is not well known (Wang et al. 2006; Dar et al. 2017). Monferrán and Wunderlin (2013) suggested the passive entry of Pb into the roots, but according to Pourrut et al. (2013), the transport of Pb into plant roots is aided by ZIP/CDF or natural resistance-associated macrophage proteins (Nramps). Lead is deposited mainly near the inner cell gap and cell wall of root cells as confirmed by Li et al. (2016) who found Pb, along with P, Cu, C, and O, as dense black granules using transmission electron microscopy. Yuan et al. (2015) also confirmed Pb compartmentalization near the cell wall, epidermis, stele and vascular tissues in the roots of *Iris lactea* by using Leadmium™ Green AM molecular probe and fluorescence microscopy. The retention of Pb in the roots is likely due to its greater affinity with pectin and carboxyl groups within the cell wall (Qiao et al. 2015). Sequestration of Pb in the vacuoles and its precipitation in intercellular spaces also lead to immobilization and retention of Pb in roots (Pourrut et al. 2013).

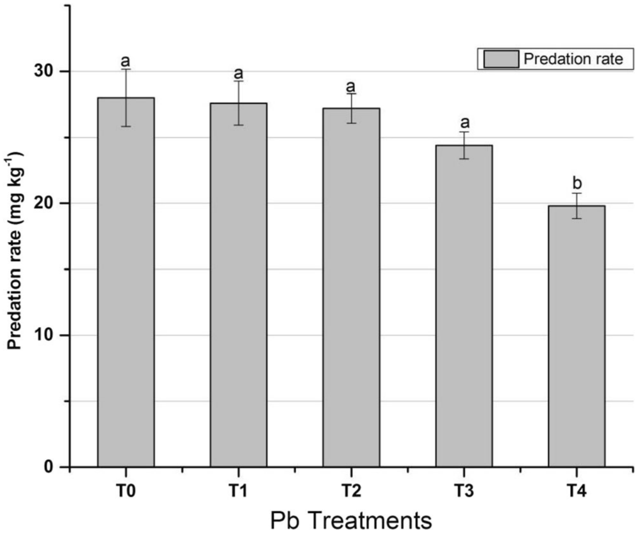

Fig. 3 Effect of various levels of Pb treatments (T0, control soil; T1, 25 mg kg^{-1}; T2, 50 mg kg^{-1}; T3, 75 mg kg^{-1}; T4, 100 mg kg^{-1} Pb treatments) on the consumption of aphids by ladybird larvae (mean ± SE; $n = 4$)

Pb transfer from root to shoot

In the present study, Pb mostly remained in the roots with a lower but still significant amount translocated from root to shoot. The TCs of Pb from root–shoot were lower than 1 (~0.54) and lower than those of soil–root. These results are in line with the findings of Liu et al. (2009), Shahid et al. (2011), Dar et al. (2015, 2017), Zhou et al. (2016) and Zhang et al. (2017), who reported the accumulation of Pb mostly in the roots of plants and only a small amount was translocated to aerial parts. Retention of Pb in roots and its lower root–shoot translocation may be due to immobilization or precipitation of Pb by negatively charged lignin and pectin or binding to mucilage uronic acids within the cell walls of roots (Arias et al. 2010; Islam et al. 2007), sequestration of Pb in the vacuoles of cortical and rhisodermal cells (Kopittke et al. 2007, Pourrut et al. 2011), accumulation of Pb in plasma membranes (Jiang and Liu 2010) or precipitation of insoluble salts of Pb in intercellular spaces (Malecka et al. 2009). In

Table 4 Variation in fresh and dry mass (mg individual^{-1}) of aphids and newly emerged adult ladybirds exposed to various application rates of Pb (mean ± SE; $n = 4$). Values with different superscript letters in each group are significantly different from each other at $p < 0.05$

Amendments	Fresh mass (mg)		Dry mass (mg)	
	Aphid	Adult ladybird	Aphid	Adult ladybird
T0	0.23 ± 0.02^{a}	32.05 ± 1.42^{a}	0.034 ± 0.003^{a}	6.82 ± 0.24^{a}
T1	0.22 ± 0.02^{a}	31.15 ± 1.27^{a}	0.032 ± 0.002^{a}	6.68 ± 0.23^{ab}
T2	0.20 ± 0.02^{ab}	30.62 ± 1.18^{a}	0.030 ± 0.002^{ab}	6.56 ± 0.16^{ab}
T3	0.19 ± 0.02^{ab}	29.55 ± 1.04^{ab}	0.029 ± 0.002^{bc}	6.43 ± 0.19^{b}
T4	0.17 ± 0.02^{b}	28.85 ± 1.05^{b}	0.027 ± 0.002^{c}	6.36 ± 0.21^{b}

T0, control soil; *T1*, 25 mg kg^{-1} ; *T2*, 50 mg kg^{-1} ; *T3*, 75 mg kg^{-1} ; *T4*, 100 mg kg^{-1} Pb treatments

addition, the endodermis, which acts as a physical barrier for ion transport through the apoplastic pathway, may also play a role in reduced Pb translocation (Kumar and Prasad 2018). Following apoplastic transport, Pb may precipitate in the endodermis due to the Casparian strip, and if transferred by symplastic transport, a major portion of it may get sequestered or excreted by the plant's detoxification systems (Kaur et al. 2013).

Pb transfer from shoot to aphid

The accumulated Pb content in the bodies of black bean aphids feeding on broad bean shoots was higher than among other phytophagous insects witnessed in previous studies (Kazimirova and Ortel 2000; Dar et al. 2015; Zhou et al. 2015; Dar et al. 2017; Zhang et al. 2017). This may be due to the higher bioavailability of Pb in the studied soil and its greater mobility in the phloem sap (Liao et al. 2006), influencing the transfer to higher trophic level (Wang et al. 2006). The TCs between shoot to aphid were higher than those of root to shoot but were below 1 (~0.85), indicating that Pb did not biomagnify in aphids (Table 2). The same results were reported by Devkota and Schmidt (2000), Zhou et al. (2015), Dar et al. (2015, 2017), and Zhang et al. (2017), also observing biominimization of Pb in grasshoppers, silk worms (*Bombyx mori*), aphids (*Lipaphis erysimi*) and mealybugs (*Dysmicoccus neobrevipes*), respectively. Moreover, elimination of considerable amounts of Pb with honeydew of aphids could have played a significant role in minimizing their Pb body burden (Table 3), as also reported by Dar et al. (2015, 2017).

Pb transfer from aphid to ladybird

The TCs of Pb between aphids and freshly emerged ladybirds were below 1 (about 0.67) and lower than those of shoot to aphids, indicating biominimization of Pb in predatory ladybirds. Our findings corroborate the findings of Dar et al. (2015, 2017) and Zhang et al. (2017). Ladybirds mainly feed for their development during 4th larval instar stage (Darshana and Abishek 2015) and hence the maximum heavy metal accumulation took place in the fourth instar (Gintenreiter et al. 1993; Dar et al. 2015, 2017). The insects are known to eliminate the accumulated toxic metals during pupation by shedding of pupal exuviae (Dar et al. 2015). In our study, a significant amount of Pb was eliminated during metamorphosis through pupal exuviae. The loss of Pb through pupal exuviae ranged from 7.12 to 11.70%. Similar Pb elimination strategies by different insect species have been reported by Zhou et al. (2015), Dar et al. (2017) and Zhang et al. (2017). Zhou et al. (2015) inferred the mechanism of Pb removal from the insect body by stating that the Pb content assimilated in the alimentary system is transferred to other organs through the epithelium tissue of the gut. Subsequently, during the process of metamorphosis, Pb associated with the alimentary system is lost due to the renewal of epithelium. The Pb which penetrates other tissues through gut epithelium is not lost during metamorphosis and therefore can be accumulated in the adult ladybird.

Effects of Pb on aphids and newly emerged adult ladybirds

The biomass (fresh and dry mass) of aphids decreased significantly ($p < 0.05$) when nurtured on broad bean plants grown on soils contaminated with Pb at higher concentrations (Table 4). The loss of dry weight in aphids may be due to reduced sucking of phloem by aphids on broad bean plants grown in soil with higher Pb levels. The Pb toxicity caused due to accumulation in aphid bodies could possibly be another reason for the reduction in dry weight (Crawford et al. 1995).

All selected doses of Pb except T4 did not have any impact ($p > 0.05$) on the consumption of aphids by predatory ladybird larvae (Fig. 3). It may be inferred that the Pb accumulated in aphid (*A. fabae*) bodies at lower concentrations were unable to produce any deterrence on their natural predator (*C. transversalis*), but the insects exposed to T4 reduced predation rate. This shows that Pb may have sub-lethal effects even in the absence of biomagnification. Therefore, it is imperative to better understand the impact that accumulated Pb may have on biological control agents so that these species can be protected and used successfully in integrated pest management programs.

Conclusion

This study examines the potential for biotransfer of Pb across a soil–plant–aphid–ladybird food chain. Significant variations in Pb concentrations were observed across the various components of the food chain. Lead biomagnification was observed for soil to root transfer whereas biominimization of Pb was observed during shoot–aphid–ladybird transfer. Elimination of Pb via the honeydew of aphids and through pupal exuviae during the metamorphosis of ladybirds was also observed. The predation rate and biomass of ladybirds revealed that the higher doses of Pb may have lethal or sublethal effects on predatory ladybirds. The potential consequences of Pb mobility in the consumer trophic levels and the ecotoxicological consequences are particularly concerning. Further physiological and biological studies are needed to investigate the above-mentioned effects.

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