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RESEARCH ARTICLE

Heavy metal exposure through artificial diet reduces growth and survival of *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract

Insect physiology is affected by the presence of toxins in the surrounding environment of insects as well as their food sources. The objective of this study was to determine the effect of heavy metal exposure to two low concentrations (50 μg/g and 150 μg/g) of lead (Pb) and zinc (Zn) through artificial diet to the larvae on biological parameters of Asian armyworm (*Spodoptera litura* Fabricius) (Lepidoptera: Noctuidae). Both Pb and Zn, even at low concentrations, had relatively high toxic effects on *S. litura* larvae ($P < 0.01$). *S. litura* larval weight and length suffered the maximum reduction when the larvae were fed on diet mixed with the high Pb concentration (150 μg/g) tested compared to the other treatments. At the same Pb concentration (150 μg/g), values of larva growth index, pupa growth index, immature growth index, standardized growth index, and fitness index were 4.66, 7.33, 7.82, 5.35, and 10.00 times lower, respectively, than those of control. At the same Zn concentration (150 μg/g), values of larval growth index, pupal growth index, immature growth index, standardized growth index, and fitness index were 5.61, 3.00, 3.04, 3.23, and 9.24 times lower, respectively, than those of control. The survival rate of *S. litura* larvae was also lower (12.5%) when the larvae were fed on diet mixed with Pb at 150 μg/g after 10 days of observation. Overall, the presence of those heavy metals in the environment, even at low concentrations, would exert an adverse impact on larvae development of this insect. From this point of view, findings could provide a basis for long-term evaluation of heavy metal risk and its impact on populations of important agricultural pests.

Keywords Asian armyworm · Bioindicator · Larvae · Lead · Pollution · Stress · Zinc

Introduction

The occurrence of heavy metals in the environment creates great concern, because metals commonly deriving from industrialization, increased vehicle use, and modern farming practices contribute to environmental pollution (Atafar et al. 2010; Hamzeh et al. 2011; Liu et al. 2014). Indeed, natural ecosystems are adversely affected by common human interventions (Qadir and Malik 2009). For example, different types of metal-based pesticides, which are widely used to suppress common pests in crops, can lead to contamination of the environment (Verkleij 1993; Gimeno-García et al. 1996; Tariq et al. 2016). Moreover, common fertilization practices can also increase heavy metal concentration in soil in the long-term (Xu et al. 2018; Zhao et al. 2018). Also, the excessive amount of toxic metals in plantation sites not only contaminates soil but also can affect food quality and safety (Sharma et al. 2006; Muchuweti et al. 2006; Sharma et al. 2008).

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Insects can accumulate heavy metals in their bodies when living in polluted areas (Heliövaara and Väisänen 1990; Zvereva et al. 2003). In those areas, insects can get into direct contact with contaminated soils in aquatic and terrestrial systems, through air pollution, and also through feeding on plants that have aggregated these materials. By feeding on insects with increased concentrations of these elements, parasites and predators can be also exposed (Vickerman et al. 2002). Metals entering the soil constitute a more lasting form of pollutants due to their accumulation in soil. Waste water from industries can contaminate growing crops when used for irrigation of vegetable crops. For example, municipal waste water is used to irrigate vegetable crops in District Sargodha of Pakistan (Ahmad et al. 2013b), which potentially causes bioaccumulation of heavy metals in biota, including food supplements, with potential harmful effects on the insect body. Heavy metals are not essential for plant growth, but they can be taken up by the roots and moved to the leaves of many plant species (Marschner 1983; Nagajyoti et al. 2010; Page and Feller 2015). In turn, several pests attack vegetable crops and can be directly or indirectly influenced by heavy metals (Heliövaara and Väisänen 1990; Maestri et al. 2010).

Insect behaviors play a significant role in the ecological interactions with plant species as well as with the abiotic environment, and therefore, these behaviors play a major role in the diversity of ecosystems (Fisher 1998; Campbell et al. 2012). Despite the crucial role of insects in most ecosystems, there is limited information on the impact of heavy metal contamination on the behavior of insects. Few insect species show tolerance against heavy metals at low levels, while most insects are negatively affected by metals, showing slower growth, development, and fertility (Xia et al. 2006). Heavy metals like Cd, Pb, and Zn showed detrimental effects on diamondback moth (*Plutella xylostella*) (Lepidoptera: Plutellidae) at concentrations near normal ranges found in plants (Coleman et al. 2005). Moreover, artificial diet with heavy metals (Zn and Pb) significantly decreased the survival and pupation rate of *Plutella xylostella* (Jhee et al. 2006), while both Cd and Pb, even at very low concentrations, had relatively high toxic effects on *Spodoptera littoralis* larvae (Eesa et al. 2017).

Asian armyworm (*Spodoptera litura* Fabricius) (Lepidoptera: Noctuidae) is an economically important insect pest in the Asia-Pacific region and cause massive damage to agricultural crops (Gao et al. 2004). In Pakistan, *S. litura* is considered as a major insect pest of many crops (Saleem et al. 2008; Ahmad et al. 2013a). Previous research showed that exposure of *S. litura* to heavy metals had various toxic effects on this pest, especially Ni (Sun et al. 2008, 2010), Pb, and Zn (Xia et al. 2005; Shu et al. 2009, 2012a, b; Kafel et al. 2014). However, the above studies examined relatively high concentrations of those metals, typically above 50 mg, whereas lower rates were not tested. We hypothesize that many agricultural fields may accumulate concentrations of Pb and Zn that are high enough to alter the physiology of *S. litura*, but pertinent studies on the topic are lacking. Nevertheless, any negative effect of heavy metals on insect growth and survival could be utilized to test dual benefits of hyper-accumulator plants in controlling insect population and cleaning contaminated soils. Moreover, relevant findings could provide a basis for long-term evaluation of heavy metal risk and its impact on populations of important agricultural pests. In the light of the above, the current study tried to evaluate the impact of Pb and Zn at two concentrations (50 and 150 μg/g) on the growth, development, and survival of *S. litura*, for which no data exist in the literature.

Materials and methods

Insect culture

The egg batches of *S. litura* were collected from a tomato field near the University of Sargodha, Pakistan. Chickpea-based artificial diet (Seth and Sharma 2002) was provided to newly hatched larvae and the culture was maintained at 25–27 °C with 60–80% relative humidity (RH). The *S. litura* adults were released in rearing cages (40 cm × 40 cm × 40 cm) and were provided with 10% sugar solutions on absorbent cotton. The eggs laid by females were taken as a starting point for the first generation, while the second instar larvae of F_2 generation (synchronized population) were used for further experiments.

Heavy metal source

The salts of Zn in the form of $ZnCl_2$ and Pb in the form of $Pb(NO_3)_2$ were procured from commercial suppliers (Chemical Reagent Co Ltd., Shanghai, China and Wenzhou Chemical Materials Plant, Wenzhou, China, respectively). All chemical reagents were of analytical grade and used as received without further purification, unless otherwise noted.

Insect bioassay

Two concentrations of each heavy metal were added to the diet as shown in Table 1. The artificial diets containing heavy metals were fed to second instar larvae of *S. litura*. There were five treatments and each treatment replicated eight times. Two larvae were tested in each replicate totaling 16 larvae per treatment. Data for larval length (cm), weight (g), and survival rate were recorded at 24-h intervals for 10 days.

The growth and fitness parameters such as larval growth index, immature growth index, pupal growth index, standardized growth index, and fitness index of *S. litura*

Table 1 Treatment layout for the evaluation of heavy metal effects on *S. litura* larvae (Pb in the form lead nitrate, Zn = in the form zinc chloride)

Treatment	Concentration (μg/g)	Artificial diet (g)
T_1 (Pb level 1)	50	250
T_2 (Pb level 2)	150	250
T_3 (Zn level 1)	50	250
T_4 (Zn level 2)	150	250
T_5 (Control)	–	250

were measured with the formulas suggested by Pretorius (1976) and Itoyama et al. (1999) as below.

$$\text{Larval growth index} = \frac{\text{Pupation (\%)}}{\text{Larval period (days)}} \tag{1}$$

$$\text{Pupal growth index} = \frac{\text{Emergence (\%)}}{\text{Pupal period (days)}} \tag{2}$$

$$\text{Immature growth index} = \frac{\text{Emergence (\%)}}{\text{Immature stages (days)}} \tag{3}$$

$$\text{Standardized growth index} = \frac{\text{Pupal weight}}{\text{Larval period (days)}} \tag{4}$$

$$\text{Fitness index} = \frac{\text{Pupation (\%) x Pupal weight}}{\text{Larval period (days) x Pupal period (days)}} \tag{5}$$

Detection of heavy metal in *S. litura* larval body

The heavy metals were detected as per method of Parven et al. (2009). The fifth instar larvae that were fed on artificial diet mixed with heavy metals were taken, washed with distilled H_2O, and dried in an oven for 48 h at 65 °C. The dried larvae were crushed to a fine powder with an electrical grinder. The digestion of the above samples was performed according to the procedure defined by Cock et al. (1976) for the estimation of Zn and Pb accumulation in larval mass. One gram of the dried samples was transferred into a 100-mL conical flask followed by the addition of 10 mL of the tri-acid mixture (HNO_3, $HClO_4$, and H_2SO_4 at 5:2:1). The flasks were heated on a hot plate until fumes disappeared and the volume was reduced to 1.5 mL indicated by the clear colorless solution. After that, 1 mL of perchloric acid was placed to each flask to cool the solution. The sample was filtered with Whatman No. 1 filter paper after cooling and then diluted up to 250 mL using deionized distilled water in a volumetric flask. Accumulation of heavy metals (Zn and Pb) was detected by atomic absorption spectrophotometer (AA-6300 Series Polarized Zeeman, Hitachi, Japan), using the specific lamp for each specific metal at Hi-Tech Laboratory, Department of Pharmacy, University of Sargodha, Pakistan. First, standard solutions were used to calibrate the instrument and then a calibration curve was created. The actual concentration was estimated in the samples by establishing calibration curves.

Statistical analysis

Data for larval length, weight, growth, and fitness indices, as well as heavy metals accumulation, were subjected to analysis of variance under a completely randomized design (CRD). The survival rate was calculated by the Kaplan-Meier test at a confidence level 95%. Means (all-pairwise comparisons) were compared with Tukey's honestly significant difference (HSD) test at $P < 0.05$. Moreover, regression analysis was used to determine the relationship of each studied variable to the rates of Zn and Pb tested. All analyses were performed using SPSS (ver. 20.0) and Minitab (ver. 16.1) software.

Results

Larval length, weight, and survival rate

Heavy metals (Pb and Zn) significantly reduced larval length ($F = 57.6$; $P < 0.01$) and weight ($F = 44.2$; $P < 0.01$) of *S. litura* in a dose-dependent manner (Fig. 1). The maximum reduction of larval length (0.54 cm) and weight (0.06 g) was found in larvae fed on diet mixed with Pb (150 μg/g) as compared with control (1.94 cm and 0.27 g, respectively) (Fig. 1). Linear regression showed that Pb exerted more toxic effect on both traits than Zn (greater slopes of Pb than those of Zn), while the effect was more severe on larval length (Table 2).

A significant difference ($\chi^2 = 10.223$; $P < 0.01$) was found in the survival of *S. litura* larvae after exposure to heavy metals compared with control. The survival rate was higher (85–100%) in control than the heavy metal exposed treatments during all sampling days (Fig. 2). However, larvae survival was significantly reduced with the application of heavy metals. The low concentrations of Pb and Zn (50 μg/g) reduced significantly survival rate from the fourth day of exposure, while the high concentrations of Pb and Zn (150 μg/g) reduced significantly survival rate even from the second day of exposure (Fig. 2). The maximum reduction of survival rate occurred after exposure to the high rates of Pb and Zn (150 μg/g) at the end of the assessment period.

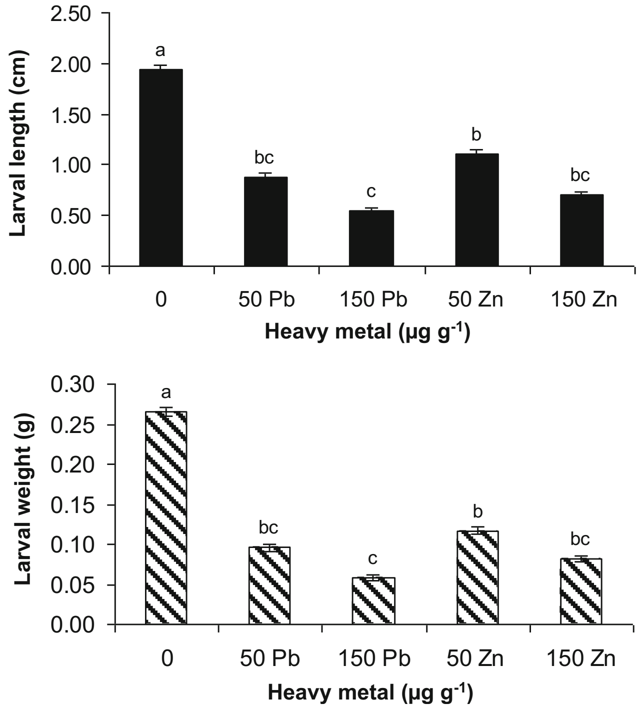

Fig. 1 Heavy metal effects on *S. litura* larvae length and weight (different letters indicate significant differences at $P < 0.05$; vertical bars indicate standard errors of the means)

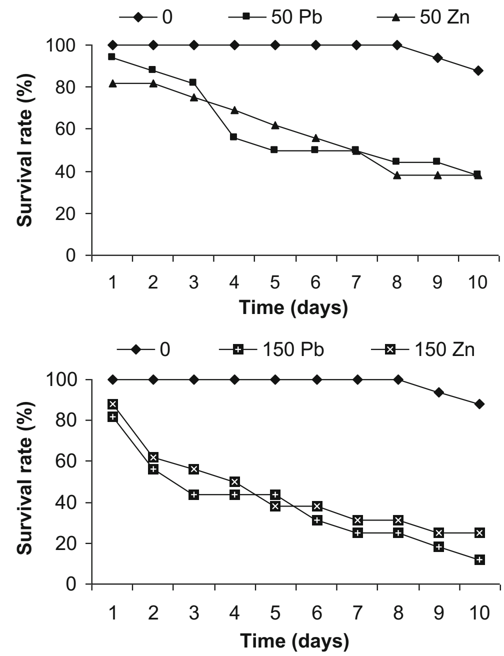

Fig. 2 Heavy metal effects on survival rate of *S. litura*

Growth and fitness indices

Heavy metals significantly affected ($P < 0.01$) larval growth, pupal growth, and immature growth of *S. litura* larvae in a dose-dependent manner (Fig. 3). The larval growth index was maximum (16.49) in control, while the lowest value (2.94) was observed when larvae were fed on Zn at 150 µg/g, which was statistically at par with Pb (3.54) when fed with the same concentration (150 µg/g). The pupal growth index of *S. litura* was maximum (15.45) in control, while the lowest value (2.11) was observed when larvae were fed on diet mixed with Pb at 150 µg/g, followed by the value 5.14 when larvae were fed on Zn at the same concentration (150 µg/g) (Fig. 3). Similarly, the immature growth index significantly decreased (1.05) in the case of Pb at 150 µg/g compared with control (8.25). Similar results were also found in the case of standardized growth and fitness index (Fig. 4). Control showed the highest values of standardized growth (0.054) and fitness index (2.33), while the lowest values (5.35 and 10.00 times lower than control) occurred with the high rate of Pb (150 µg/g). Linear regression showed that Pb exerted more toxic effect on most traits (greater slopes of Pb than those of Zn), except from LGI and FI, where Zn was found more toxic (Table 3). The effect of heavy metal exposure was more severe on LGI and PGI as compared with the other indices (Table 3).

Table 2 Linear regression ($Y = ax + b$) coefficients for the studied parameters

Dependent variable (*Y*)	Slope (*a*)	Intercept (*b*)	R^2
Larval length [Zn]	−0.621	2.488	0.962
Larval length [Pb]	−0.699	2.517	0.918
Larval weight [Zn]	−0.092	0.339	0.885
Larval weight [Pb]	−0.104	0.348	0.882

Detection of heavy metal in larval body and feces

Heavy metal exposure significantly affected ($P < 0.01$) Zn and Pb accumulation in fifth instar larval body of *S. litura*. Atomic absorption spectrophotometry indicated that 2 µg/g of either $ZnCl_2$ or $Pb(NO_3)_2$ were found in the body of untreated larvae (control) (Table 4). However, 120 µg/g of $Pb(NO_3)_2$ was detected in larvae feeding on either concentration of $Pb(NO_3)_2$, while the $ZnCl_2$ content in larvae feeding on either concentration of $ZnCl_2$ was zero. The $Pb(NO_3)_2$ content was zero in larvae feeding on both concentrations of $ZnCl_2$, but $ZnCl_2$ content was 120 µg/g in those larvae (Table 4). Similarly, heavy metal exposure significantly affected accumulation Zn and Pb ($P < 0.01$) in feces of fifth instar larvae of *S. litura*. Quantities of 2.5 µg/g of $ZnCl_2$ and 1.5 µg/g of $Pb(NO_3)_2$ were found in feces of untreated larvae (control) (Table 4). However, a quantity of 200 µg/g of $Pb(NO_3)_2$ was detected in feces of larvae feeding on both concentrations of $Pb(NO_3)_2$, while $ZnCl_2$ content was zero. Conversely, $Pb(NO_3)_2$ concentration was zero in feces of larvae feeding on both concentrations of $ZnCl_2$, but $ZnCl_2$ content was 200 µg/g in feces of those larvae (Table 4).

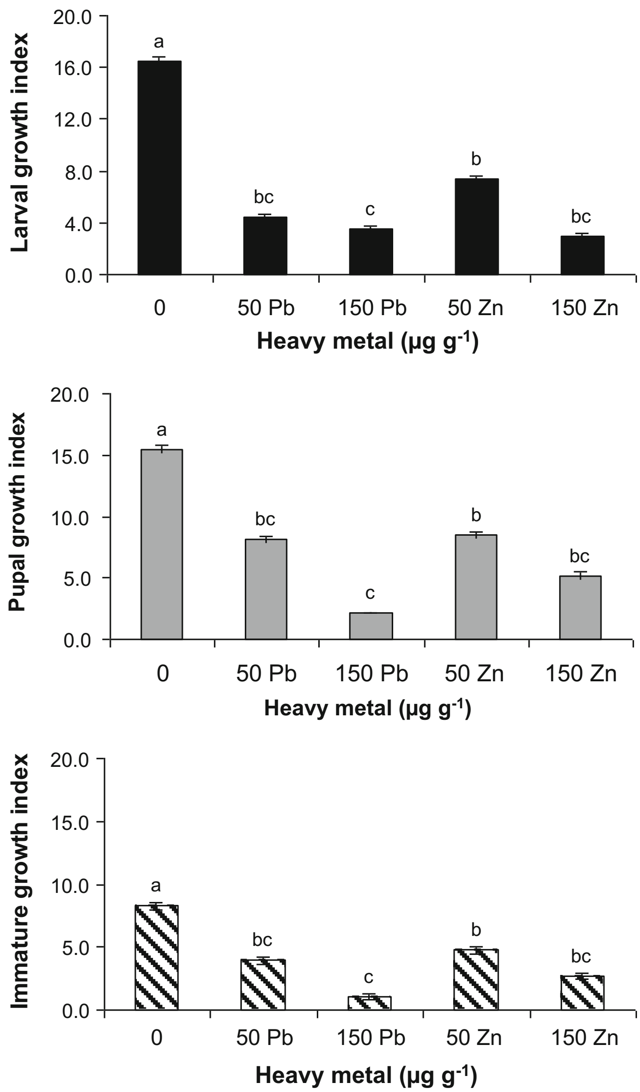

Fig. 3 Heavy metal effect on *S. litura* growth indices (different letters indicate significant differences at $P < 0.05$; vertical bars indicate standard errors of the means)

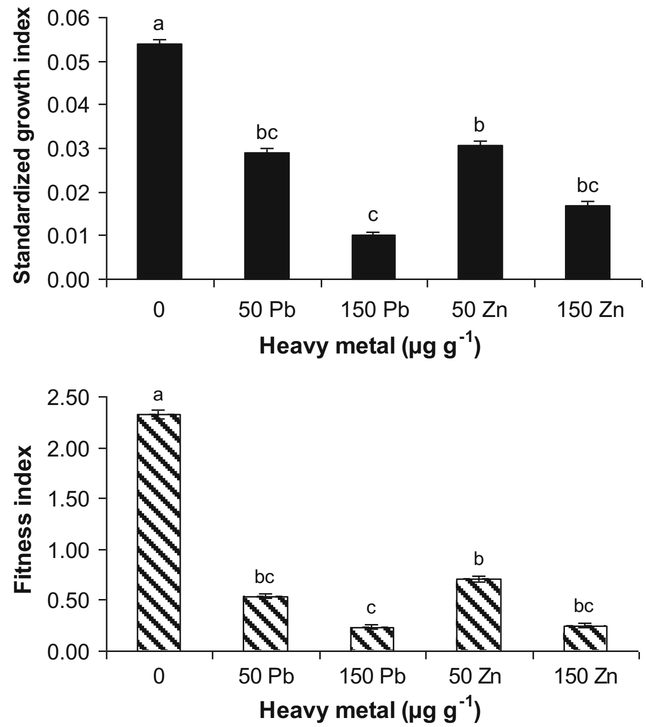

Fig. 4 Heavy metal effects on standardized growth index and fitness index of *S. litura* larvae (different letters indicate significant differences at $P < 0.05$; vertical bars indicate standard errors of the means)

Discussion

Many agricultural fields may accumulate concentrations of Pb and Zn that are high enough to alter the physiology of *S. litura*, but pertinent studies on the topic are lacking. In previous research, artificial diet with Zn and Pb significantly decreased the survival and pupation rate of *Plutella xylostella* (Jhee et al. 2006). In the current study, giving artificial diet enriched with low rates of Zn or Pb to *S. litura* showed a significant dose-dependent effect on growth and development parameters of this insect. Several researchers reported harmful effects of Zn on insect growth and development (Coleman et al. 2005; Sharaby et al. 2011; Bahadorani and Hilliker 2009; Al-Dhafar and Sharaby 2012; Kazemi-Dinan et al. 2014). However, Stolpe and Müller (2016) reported an increased aphid growth when fed on Zn-enriched diets, whereas Cd reduced the survival of aphids. In line with the results of our study, a recent study (Eesa et al. 2017) found that both Cd and Pb, even at very low concentrations, had relatively high toxic effects on *S. littoralis* larvae, with Cd being more toxic than Pb. Moreover, many morphological and structural changes were noticed in larvae and pupae of *Drosophila melanogaster* after 48 h of Pb acetate application (Haq et al. 2011).

Zn is an important element for animal nutrition (Maret 2005), but can negatively affect growth and development of organisms, especially insects, if its concentration exceeds

Table 3 Linear regression ($Y = ax + b$) coefficients for the studied parameters

Dependent variable (Y)	Slope (a)	Intercept (b)	R^2
Larval Growth Index [Zn]	− 6.773	22.479	0.961
Larval Growth Index [Pb]	− 6.474	21.120	0.804
Pupal Growth Index [Zn]	− 5.151	19.987	0.959
Pupal Growth Index [Pb]	− 6.669	21.885	0.996
Immature Growth Index [Zn]	− 2.770	0.339	0.977
Immature Growth Index [Pb]	− 3.597	11.606	0.986
Standardized Growth Index [Zn]	− 0.018	0.071	0.978
Standardized Growth Index [Pb]	− 0.022	0.074	0.993
Fitness Index [Zn]	− 1.040	3.175	0.903
Fitness Index [Pb]	− 0.049	3.132	0.856

Table 4 Detection of heavy metals (mean ± SE) in fifth instars of *S. litura* larval body and their feces

Heavy metals	CON (μg/g)	Larval body		Feces	
		Zn	Pb	Zn	Pb
Lead nitrate	50	0.0 b	120.0 ± 0.9129 a	0.0 b	200.0 ± 0.912 a
	150	0.0 b	120.0 ± 1.7795 a	0.0 b	200.0 ± 1.779 a
Zinc chloride	50	120.0 ± 1.5811 a	0.0 b	200.0 ± 1.825 a	0.0 b
	150	120.0 ± 2.4496 a	0.0 b	200.0 ± 1.779 a	0.0 b
Control		2.0 ± 0.4082 b	2.0 ± 0.7071 b	2.5 ± 0.6465 b	1.5 ± 0.288 b

Different letters within each column show significant different among treatments at $P < 0.05$

CON concentration

physiological limits. As reported in the literature, Zn in insect food can destroy the midgut structure and influence the processes of digestion and absorption of food (Al-Dhafar and Sharaby 2012), thus causing starvation effects and reducing larval growth and survival. Reduction in growth and development due to Pb-enriched diet might be due to Pb toxic effects on the body of larvae, which decreases larval growth and development (Safaee et al. 2014). Locusts fed on food enriched with coumarin and Zn showed aversion to Zn that was developed by a post-ingestive mechanism involving associative learning (Behmer et al. 2005). Oxidative stress causing a consistent weakness of the larvae by starvation was reported as the mechanism of acute toxicity of metals on larvae, which probably decreased survival of the larvae (Leonard et al. 2004), with mortality rate being high especially in young larvae. A high mortality rate in young larvae was also found in other Lepidopterous insects and beetle larvae (Lapointe et al. 2004). Diet enriched with Zn or Pb increased mortality of *Plutella xylostella* larvae (Jhee et al. 2006), mortality of three herbivore species specialists on Brassicaceae (i.e., *Pieris napi*, *Athalia rosae*, and *Phaedon cochleariae*) (Kazemi-Dinan et al. 2014), and mortality of the generalist aphid *Myzus persicae* (Stolpe and Müller 2016).

Heavy metal contamination in our environment has become a major problem (Sun et al. 2007). These metals are immobilized in soil and can remain for long at the same place; thus, they can enter into organisms through the food chain (Qin et al. 2010). Previously, Lepidopterous insects have been used as bioindicators of environmental pollution near industrial and urban areas due to increased levels of heavy metals and carbon dioxide concentration (Azam et al. 2015). The occurrence of Cu, Cd, Fe, Ni, and SO_4^{-2} ions and other materials used in fertilizers were studied in pupae of various Geometridae and Noctuidae species (Heliövaara and Väisänen 1990) and by monitoring the different life cycle and mortality rate in hatched larvae of butterflies (Nymphalidae) that feed on plants subjected to high carbon dioxide concentration (Fajer et al. 1989). The ecology, morphology, and physiology of insects are greatly affected by the presence of toxins, including heavy metals like Zn, Co, Ni, Pb, Cd, and Cr in their environment and food sources, as previously reported in earlier studies (Sorsa and Pfeifer 1973; Jeantet et al. 1977; Martoja et al. 1983), while the use of some insect groups as heavy metal indicators is examined up to nowadays (Nummelin et al. 2007; Azam et al. 2015).

Feeding efficiency is the ability of insects to ingest the food to the best of its potential. Our study demonstrated the effect of heavy metals on the feeding indices of *S. litura* larvae. The larval weight and length were reduced dramatically when the larvae were fed on diet mixed with the high concentration of either heavy metal compared with control (artificial diet only). A significant negative effect of Zn was previously found on larval mortality, pupal stage, and adult emergence of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Popham and Shelby 2006). Similarly, Baghban et al. (2014) observed reduced growth and mounting rate of cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) due to increased glycogen level by $ZnCl_2$ application. Kafel et al. (2014) also noted that Zn decreased *S. exigua* larval weight.

The growth parameters of *S. litura*, such as larval growth index, pupal growth index, and immature growth index as well as standardized growth index and fitness index, were the lowest when larvae were fed on diet mixed with the high concentrations of either Pb or Zn. A previous study showed that larval survival rate, pupation percentage, and adult emergence were also decreased significantly with increasing the amount of heavy metal stress (Sun et al. 2007). Furthermore, *Drosophila melanogaster* (Diptera: Drosophilidae) larval survival rate was decreased significantly after feeding on diet mixed with Cd (Shirley and Sibly 1999). Comparatively, the high concentration of Pb reduced larval length and growth to the maximum extent compared with Zn. Our results keep up to those of Ying-Hua et al. (2012) who noted that Pb caused a significant effect on *S. litura* larval growth and reproduction. With an increase in Pb concentrations, body weight and survival rate of the larvae at different developmental stages (larvae, pupae, and adult) were reduced. The survival rate of *S. litura* larvae was the lowest when the larvae were fed on diet mixed with the high concentrations of either Pb or Zn, while the highest survival rate was observed in control. Our findings are in accordance with those of Popham and Shelby (2006) who reported that Zn negatively affected survival of immature stages of *H. virescens* and about 80% of the larvae failed to convert into pupae at 60 ppm concentration.

The accumulation of heavy metals in the insect body may hinder growth and development of these organisms. After application of Pb, the accumulation of heavy metals in larval body and feces was in the order Pb > Zn, while after application of Zn, the opposite trend (Zn > Pb) was observed. However, the accumulation of both heavy metals was found to be higher in feces compared to the larval body. According to Augustyniak et al. (2006), the excessive amount of Zn accumulated in grasshopper *Chorthippus brunneus* (Orthoptera: Acrididae) brain caused impairment in DNA structure. According to Huang et al. (2012), the feeding of *S. litura* on artificial diet mixed with Cu at 50 mg/kg may result in Cu accumulation in later larval stages (fourth and fifth instar), which provoked low rate of survival in the later larval instars during pupation period as well as adult emergence, eventually leading to other negative impacts on fertility and fecundity. Such accumulation of heavy metals may retard growth and development of insects. Our results clearly suggest that Pb and Zn, even at low rates, have potential to affect insect growth and survival, but the mechanism of these effects is still unknown. Therefore, future studies, probably at molecular level, might be useful towards documentation of the toxic mechanisms of heavy metals on this insect.

Conclusion

Understanding modification of insect behavior after exposure to heavy metals is necessary to assess the importance of heavy metal contamination. The current study evaluated the negative effects of low rates of Zn and Pb exposure through artificial diet on *S. litura*. Overall, the presence of those heavy metals in the environment, even at low concentrations, is capable of exerting a significant adverse impact on larval development of this insect. Findings highlight the threat to insect lives in the industrial area of Pakistan, where Pb and Zn concentrations are increasing and call upon measures for lessening environmental pollution in all levels (soil, air, and water) by enforcing implementation of pollution control laws. Moreover, findings could provide a basis for long-term evaluation of heavy metal risk and its impact on populations of important agricultural pests.

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