Ecotoxicology and Environmental Safety 143 (2017) 289–295

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Transfer of lead (Pb) in the soil-plant-mealybug-ladybird beetle food chain, a comparison between two host plants

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ARTICLE INFO

Keywords:
Bio-transfer
Lead
Bio-minimization
Metamorphosis
Contamination

ABSTRACT

Contamination of soil with heavy metals has become an issue of concern on global scale. This study investigates the translocation of lead (Pb) along the soil - plant (eggplant and tomato) - mealybug (*Dysmicoccus neobrevipes)* - ladybird beetle (*Cryptolaemus montrouzieri)* food chain. Soil amendments used for this study were adjusted to 0, 25, 50 and 100 mg/kg of Pb (w/w). The results revealed significantly higher transfer of Pb in tomato when compared to eggplant. Bio-magnification of Pb (2–4 times) was observed for soil - root transfer whereas Pb was bio-minimized in later part of food chain (shoot – mealybug - ladybird transfer). A dose dependent increase in transfer of Pb across the multi-trophic food chain was observed for both host plants. A decrease in coefficients of Pb transfer (from root - shoot and shoot - mealybug) was observed with increase in Pb concentrations. Our results also showed removal of Pb from the bodies of ladybird beetle during metamorphosis. Further studies are required to explain the mechanisms or physiological pathways involved in the bio-minimization of Pb across the food chain.

1. Introduction

Heavy metals constitute a major part of the Earth's crust; however, contamination of soil with heavy metals has become an issue of concern on global scale. Ecosystem is being constantly contaminated with heavy metals as a result of different activities like smelting, metalliferous mining, injudicious use of agricultural chemicals (like fertilizers) and compounds released from these sources (Anjum et al., 2016a). Because of higher affinity with organic matter, heavy metals can accumulate in soil for longer periods which can affect the growth, reproduction and community structure of living organisms and often results in oxidative damage (Santorufo et al., 2012; Anjum et al., 2016b). Heavy metals like copper (Cu) and zinc (Zn) are essential elements for the growth of different living organisms, while lead (Pb) is a non-essential element with high toxicity (even at low concentrations) against humans as well as other organisms of ecosystem. Lead is absorbed by plants from soil through roots (Ashraf and Tang, 2017) and the absorbed Pb is further transferred to herbivores as these organisms feed on aerial parts of plants (Wang et al., 2006). Therefore detailed insight on transfer of Pb across a food chain is required to know its effects on an ecosystem (Green and Walmsley, 2013).

A clear knowledge about the effects of Pb on general processes of communities as well as specific species is required to observe the effects of increased Pb exposure on different processes of an ecosystem (Gorur, 2006). As one of the classic examples, the soil - plant - herbivore - predator food chain can offer information about the effects of increased Pb concentrations on model ecosystem (Dar et al., 2015). Plants normally serve as a route of Pb transfer from soil to herbivores while the trophic level of herbivores in Pb contaminated ecosystems serve as a source of Pb accumulation as well as transfer to organisms at higher trophic levels (Devkota and Schmidt, 2000). Insect predators are important component of an agro-ecosystem because of their role in pest management (Wang et al., 2017). The feeding of insect predators on food/prey contaminated with Pb can influence their growth and reproduction which in turn can inhibit the beneficial role of insect predators in agro-ecosystem (Dar et al., 2015). Although phytophagous as well as predatory insects perform key ecosystem functions by transferring energy and contaminants to higher trophic levels. A few

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http://dx.doi.org/10.1016/j.ecoenv.2017.05.032
Received 22 November 2016; Received in revised form 17 May 2017; Accepted 19 May 2017
Available online 27 May 2017
0147-6513/

studies are available on the accumulation and interaction of trace metals in these organisms. Green et al. (2003) and Dar et al. (2015) have compared the extent to which different heavy metal are bio-transferred in the plant - aphid - ladybird food chain. Apart from the above mentioned research contribution, no work has been reported on other species of plants, phytophagous insects and their predators which can have similar link in food chain or can explain the possible variations as well as interactions involved in the food chain.

The capacity of Pb uptake, transport and accumulation of plants can vary between different plant species (Akinci et al., 2010). Some plants are hyper-accumulators which can take up and accumulate higher metal concentrations while some are hypo-accumulators as they can accumulate few metals to their tissues (Wang et al., 2006). The accumulation of Pb can further vary with different organs of a plant species (Akinci et al., 2010). The changes in the abilities of host plants for Pb accumulation can further influence the transfer of Pb to herbivorous as well as predatory insects (Zhou et al., 2015). To date, no study is available on transfer and accumulation of Pb in pink hibiscus mealybug (*Dysmicoccus neobrevipes*), which is a serious pest of different crops (Qin et al., 2011).

Cryptolaemus montrouzieri Mulsant, known as mealybug ladybird or mealybug destroyer, consumes major portion of food required for its development during 4th larval instar (Reddy et al., 1991). Feeding on prey (mealybugs) with Pb accumulation in its body can also increase the Pb contents in the body of *C. montrouzieri*. The Pb accumulated in 4th instar *C. montrouzieri* larvae can be transferred to adult stage. No research work has been reported to date which can explain the influence of metamorphosis on Pb concentration in *C. montrouzieri* adults.

Therefore, current studies were performed to calculate the extent of Pb transfer cross a soil - plant - mealybug - ladybird beetle food chain with two different host plants (tomato and eggplant). These plants were selected for experiments because of their adaptability to widespread climatic conditions, larger biomass and their use as food by humans. Efforts were also made to observe the regulation of the Pb concentrations by newly emerged adults of *C. montrouzieri* through sequestration in pupal exuviae. The main objectives of this work were to observe: a) the influence of different host plants on the amount of Pb transfer from soil in a plant – herbivore - predator food chain; b) whether transferred Pb was bio-accumulated or bio-minimized at different levels of a food chain, and c) any sub-lethal effects of Pb transfer across the food chain through the assessment of changes in dry weights of different organisms.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used in this research work were analytical grade. All the chemicals were supplied by Tianjin Kemiou Chemical Reagent Co., Ltd., China.

2.2. Insect cultures

Dysmicoccus neobrevipes cultures were maintained on tomato plants following the Qin et al. (2011) at Key laboratory of Bio-Pesticide innovation and application of Guangdong Province, South China Agricultural University, Guangzhou. Briefly, 100 reproductively active females of *D. neobrevipes* were used to start colonies and reared for two generations. The rearing conditions were set at 26 ± 1 °C, 75 ± 10% R.H., and a photoperiod of 14 h of light: 10 h of darkness. Gravid females from the second generation were placed in a rearing-box (9.0 cm × 6.3 cm × 5.0 cm) for egg production and the newly hatched nymphs were used for further experiments.

Cryptolemus montrouzieri adults were collected from the experimental area of the South China Agricultural University, Guangzhou, Guangdong, China in 2015. The beetles were reared for several generations on *D. neobrevipes* under laboratory conditions at 26 ± 2 °C, 70 ± 10% R.H. and a photoperiod of 14 h of light: 10 h of darkness.

2.3. General experimental design

Sandy loam soil collected from agricultural field of South China Agricultural University was divided in four equal parts (Merrington et al., 1997a, 1997b). Heavy metal salt lead nitrate ($PbNO_3$) was used in this study for Pb contamination of soil. Lead nitrate was dissolved in the water to make a stock solution of Pb having a concentration of 2000 ppm. Three parts of soil were amended with stock solution of $PbNO_3$ to prepare lead concentrations of 25, 50 and 100 mg/kg of soil. The remaining fourth part was used as untreated control following the method of Deng et al. (2004). The soil and Pb solutions were thoroughly mixed and filled in plastic pots (Ø 25 cm). The soil in pots was kept at field capacity and samples were collected for analyzing different properties of soil. All the experiments were replicated four times.

Tomato or eggplant seedlings were sown in each pot (one seedling per pot), and the pots were placed in a glasshouse following a completely randomized design at 26 ± 1 °C and a photoperiod of 16 h of light: 8 h of darkness. Plastic plates were placed under each pot to avoid leaching of chemicals. De-ionized water was added to the pots as per seedling requirement. During the flowering stage, mealybugs (300 individuals) reared in the laboratory were transferred to each plant. Mealybugs were allowed to feed on host plants for 3 weeks. All the mealybugs were collected from each plant after 3 weeks of feeding. The collected mealybugs were divided into two groups. One group was used for feeding the predatory beetle *C. montrouzieri* while the other group was used for Pb analysis. At the same time, the plants were also sampled for heavy metal analysis.

The mealybugs harvested from pot cultures of tomato and eggplants were used for feeding experiments following the method of Green et al. (2003). Briefly described as follows: 4th instar larvae of *C. montrouzieri* (32 individuals) separated from laboratory culture were divided into eight equal treatment groups and each individual was randomly assigned to mealybugs harvested from pot cultures of tomato and eggplants in Petri dishes (Ø 9 cm) lined with a moist filter paper. The whole experimental setup was incubated at 25 ± 2 °C and 16:8 L/D period. Fresh mealybugs were added to the Petri dishes for larval feeding until pupation. The amount of mealybugs consumed by ladybird larvae was recorded every 24 h. The adult beetles were weighed on emergence and then placed at −20 °C until Pb quantification.

2.4. Sample preparation and quantification of Pb contents

Soil samples collected (in triplicate) from the top (15 cm) of each pot, were air dried, crushed and passed through fine sieve (2 mm mesh). Total nitrogen and organic carbon contents were determined by following Dar et al. (2015). The pH meter (Metler Toledo-Fe20) was used for measuring the pH of soil samples from a soil water suspension (1:2.5 w/v).

Lead was extracted from soil samples (one gram) through digestion with 20 ml of HNO_3/H_2O_2 mixture (6:1) at 80 °C. After complete digestion, the solution was allowed to cool down followed by filtration through Whatman filter paper No# 42. The filtered solution was diluted to 50 ml with double distilled water (ddH_2O). The extractable Pb in different soil fractions was obtained by following Lindsay and Norvell (1978).

Plants (tomato and eggplant) harvested from each pot were washed with tap water to remove the adhering soil, then two times with ddH_2O. Roots and shoots were separated from each plant. The root and shoot samples were dried in an oven at 70 °C for 72 h. The dried samples (0.3 g) were digested with 10 ml of HNO_3/H_2O_2 (6:1) at 80 °C until the solution was completely clear. After digestion, the solution was allowed