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Lead in the soil–mulberry (*Morus alba* L.)–silkworm (*Bombyx mori*) food chain: Translocation and detoxification

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HIGHLIGHTS

- Biomagnification of Pb in the soil–mulberry–silkworm system was not observed.
- Roots sequestered most of the Pb, most of the Pb in leaves bound to the cell wall.
- Excretion and homeostasis protect silkworms from Pb stress.
- Detoxification in mulberry–silkworm regulates Pb transfer along the food chain.
- Pb tolerance of mulberry and silkworm indicates the bioremediation potential.

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ABSTRACT

The translocation of lead (Pb) in the soil–mulberry–silkworm food chain and the process of Pb detoxification in the mulberry–silkworm chain were investigated. The amount of Pb in mulberry, silkworm, feces and silk increased in a dose-responsive manner to the Pb contents in the soils. Mulberry roots sequestered most of the Pb, ranging from 230.78 to 1209.25 mg kg^{-1}. Over 92% of the Pb in the mulberry leaves was deposited in the cell wall, and 95.29–95.57% of the Pb in the mulberry leaves was integrated with oxalic acid, pectates and protein, and it had low bioavailability. The Pb concentrations in the silkworm feces were 4.50–4.64 times higher than those in the leaves. The synthesis of metallothioneins in three tissues of the silkworms was induced to achieve Pb homeostasis under Pb stress. These results indicated the mechanism involved in Pb transfer along the food chain was controlled by the detoxification of Pb in different trophic levels. Planting mulberry and rearing silkworm could be a promising approach for the remediation of Pb-polluted soils due to the Pb tolerance of mulberry and silkworm.

1. Introduction

Lead (Pb) is a non-essential and toxic trace element in the environment. The soils in some locations are contaminated by Pb due to anthropogenic activities (Stafilov et al., 2010; Jin et al., 2015). Pb pollution in soils is covert, persistent and non biodegradable. Soils polluted by Pb could be a source of contamination for water and air (Laidlaw et al., 2012). Previous studies indicated that Pb was accumulated in the ecosystem through the food chains and posed a great risk to the environment and to human health (Lopes et al., 2011). However, previous studies regarding Pb transfer in terrestrial ecosystems found that the Pb concentration in higher plant or higher animals was lower than that in polluted soils (Notten et al., 2005; Roodbergen et al., 2008), even though the Pb concentrations steadily declined with increasing trophic levels along a soil–plant-insect-chicken food chain at a contaminated area (Zhuang et al., 2009). It is concluded from the previous studies that metal biomagnifications are not generally applicable to all environmental ecosystems and that accumulation in the food chain varies depending on the metal concentration, biological species and trophic level (Wang, 2002). Therefore, there is an urgent need to explore both the translocation of Pb in a soil ecosystem and the remediation of Pb-polluted soils (Peralta-Videa et al., 2009; Cao et al., 2011).

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Recent research suggested that organisms might induce a whole suite of tolerance mechanisms involving metal detoxification to cope with metal stress (Shao et al., 2010). It is increasingly accepted that the chemical speciation of non-essential metals influences the internal metal detoxification of organisms and the trophic transfer of metals along a food chain (van Gestel, 2008). It has been documented that heavy metals with inorganic and water-soluble forms in plants have higher activities (Wang et al., 2008) that enable them to be easily transferred to the next trophic level. Compared to plants that are sensitive to heavy metals, tolerant plants usually accumulate more non-essential metals in the cell wall and vacuoles to prevent them from injuring the more sensitive cell metabolic sites. The non-essential metals in the cell wall often are integrated with pectates and protein, in addition to becoming water insoluble (Wu et al., 2005; Weng et al., 2012). For animals, on the one hand, the intake of non- essential metals can be decreased by the strategy of "avoidance" actions (Zidar et al., 2004; Lukkari and Haimi, 2005), and on the other hand, a detoxification process involving "excretion" and "homeostasis" actions can be used to cope with metals stress (Ahearn et al., 2004). Thus, a mechanistic understanding of the trophic transfer of toxic metals benefits from the detoxification of organism; however, only a few studies have examined this process.

Additionally, a considerable number of studies proposed that the use of some hard woody plants can be an alternative for the removal or stabilization of metals from contaminated soils (Pulford and Watson, 2003). Those woody plants with a moderate capacity to accumulate heavy metals in the aerial parts possess characteristics of rapid growth, deep and extensive root systems and high biomass production (Unterbrunner et al., 2007; Shukla et al., 2011). Similarly, mulberry exhibits the potential for remediating metal-polluted soils. Furthermore, mulberry, which is found from the tropics to the temperate regions and from sea level to altitudes as high as 4000 m, has superior attributes of high adaptability and wide distribution (Ercisli and Orhan, 2007). Previous studies identified that mulberry exhibits tolerance to many metals (Ashfaq et al., 2009; Zhao et al., 2013). Prince et al. (2001) commented on the mulberry–silkworm food chain as being a template to assess heavy metal mobility in terrestrial ecosystems. To date, few articles have reported in detail on the transfer of Pb in the soil–mulberry–silkworm system, on the Pb detoxification mechanisms of mulberry and silkworm, and on the influence of the latter to the former.

Thus, the specific aims of this study were to (i) determine the suitability of mulberry–silkworm as an alternative method to the soil remediation of Pb pollution through an investigation of the accumulation and translocation of Pb in a soil–mulberry–silkworm system and to (ii) elucidate the underlying mechanisms controlling the trophic transfer of Pb along this food chain based on the identification of the process of Pb detoxification in mulberry and silkworm involving sub-cellular distribution and the chemical forms of Pb in mulberry leaves, the excretion of Pb and metallothionein level in silkworm.

2. Materials and methods

2.1. Soil preparation, plant materials, and animal materials

Soil for the pot experiment was collected from a cleaning field in Zhangzhou, Fujian province, China, and was air-dried and then ground with a wooden roller to pass through a 2-mm sieve. The general properties of the soil are presented in Table S1 (see supplementary material). The initial soil was incubated with 0, 200, 400, and 800 mg Pb kg^{-1} added in the form $Pb(NO_3)_2$. The soils were submitted to two wetting and drying cycles to ensure that the transformation of Pb added as solutions in the soil could achieve equilibrium and better contact the different components of the soil. After approximately two months, NPK fertilizer was added to the soils in the form of ammonium sulfate (0.68 g kg^{-1} of soil), super phosphate (0.35 g kg^{-1} of soil) and muriate of potash (0.09 g kg^{-1} of soil) and homogenized. Ten kilograms of the prepared soil was added to each pot (26 cm in upper diameter and 28 cm in height). Three pots were replicated for all the Pb treatments.

The one-year-old mulberry plant stocks (var. nongsang 14), which was a common breed and planted widely in silkworm breeding areas of China, used in this study were purchased from commercial market. Two plants were planted in each pot in May and irrigated with tap water. All the pots were placed randomly on outdoor grassland, and the plants grew under natural conditions. After three months (from May, 2013 to July, 2013), the silkworm breeding experiments were performed and some plant samples were collected for other experiments and analysis.

The hybrid silkworm used in this study was named Qingfeng × Mingyue, whose spawns were from the silkworm spawn station in Huzhou, Zhejiang Province, China. All the larvae were reared on fresh and uncontaminated mulberry leaves until their fourth molt. At the beginning of the fifth instar, 48 healthy larvae of uniform size were isolated and divided into 4 groups that fed on the mulberry leaves richened with Pb from the treatment soils. After five days, six larvae from each treatment were selected to be individually weighed and died in liquid nitrogen, and then were stored at −80 °C until analysis. The other silkworms of each treatment were allowed to grow normally and complete their life stages. The cocoons and the adults were collected, washed with distilled water and dried in an oven at 50 °C to a constant weight. Subsequently, these samples were used for analysis.

2.2. Sub-cellular distribution and the chemical forms of Pb in the leaves

To determine the Pb contents at the subcellular level of the plant leaves from each treatment, differential centrifugation was used to separate the Pb, following the methods reported by Weigel and Jäger (1980) and Wang et al. (2009).

The extraction of the chemical forms of Pb in mulberry leaves was performed according to the method described by Weng et al. (2012) and Li et al. (2013).

2.3. Chlorophyll extraction and determination

The Chlorophyll in mulberry leaves were extracted by ethanol, the concentration was determined by spectrophotometry. The detail procedure is in Section S1 (see supplementary material).

2.4. Extraction and determination of metallothioneins (MTs)

The detailed information is supplied in Section S2 (see supplementary material).

2.5. Pb concentration determination

Four chemical forms of Pb in soils were extracted according to the modified BCR procedure (Rauret et al., 1999). All of the soils, plant materials, sub-cellular distribution fractions (except the soluble fraction), the residuals from the extraction of chemical forms, and animal materials were digested with a mixture of HNO_3–HF–$HClO_4$ (3:1:1 *v*/*v*) at 160 °C for 6 h. The digested mixture was cooled to room temperature and diluted to 10 mL with super purified water. Finally, the concentrations of Pb in all of the soluble samples were directly determined by ICP-AES. The quality control