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The physiological response of *Mirabilis jalapa* Linn. to lead stress and accumulation

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ABSTRACT

Smelters, metal refineries and mining operations have all been considered as major sources of metal release into the environment. As a highly toxic and cumulative poison, once in the environment Pb is difficult to remove and can adversely affect human health. *Mirabilis jalapa* Linn. (The Marvel of Peru) is a fast growing plant that shows potential for phytostabilization of Pb contaminated soils. Fourier Transform Infrared (FTIR) spectrometry was adopted to detect physiological changes in the chemical composition of *M. jalapa* exposed to six different concentrations of Pb in solution (0, 50, 100, 200, 500 and 1000 μmol L^{-1} Pb). Results indicated that biomass was reduced in plants grown in Pb treatments compared to controls, although *M. jalapa* grew typically well at the greatest concentration, 1000 μmol L^{-1}. The concentration of Pb in plant tissues occurred in the order roots > leaves > stems, with a translocation factor of less than 0.04. The absorbance of dominating bands near 3420, 2920, 1610 and 1060 cm^{-1} firstly increased but then declined in root tissues; the bands respectively corresponding to organic acids, carbohydrate, protein and amino acids. However, no obvious changes were observed in leaves and stems. The results suggest that *M. jalapa* can reduce transportation of Pb from roots to shoots, subsequently preventing Pb toxicity in shoots.

1. Introduction

Lead (Pb) is one of the main heavy metal pollutants in the environment. With the development of modern industry, Pb presents a marked increase in the environment due to mining growth, over-application of chemical fertilizers and vehicle exhaust emissions (Pietrzykowski et al., 2014). As a non-essential element for plants, Pb induces metabolic disorders, inhibits growth and development, and following excessive exposure, even death. Furthermore, Pb threatens human health through food chain transfer. Therefore, treatment of soil contaminated by Pb has become a priority (Li et al., 2013).

Smelters, metal refineries and mining operations have all been considered as major sources of heavy metal release into the environment (Yu and Gu, 2008). Mine tailings in particular are difficult to manage due to heavy metal contamination, which makes plant establishment difficult as a result of poor physical soil structure and elevated metal toxicity (Xue et al., 2016a; Zhu et al., 2016). Establishment of plant tolerance can reduce heavy metal bioavailability (Van der Ent A et al., 2012; Xue et al., 2016b).

Plants can be classified into three main groups according to metal uptake characteristics: (i) Excluders, restrict translocation. (ii) Index plants, uptake and translocation reflect soil metal concentrations. (iii) Accumulators, plants actively concentrate metals in their tissues. There is evidence to suggest that higher plants have mechanisms to protect themselves from metal toxicity. These mechanisms include metal sequestration using organic compounds (metal-binding polypeptides), subcellular compartmentalization (generally removal of metals to the cell vacuole), active metal efflux (excreting metals by active pumps) and organic ligand exudation (organic molecules exuded by root cells) (Komal et al., 2015).

Two mechanisms for heavy metal tolerance in plants have been proposed. The first is exclusion, meaning that heavy metals are absorbed by plants but then subsequently detached through active transport or aging of organs *in vitro* to make them discharge (Kiran and Thanasekaran, 2011). The second mechanism is cumulative, whereby the heavy metal becomes non-bioactive following detoxification. Examples include Pb precipitation on cell walls of

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rice which prevent excessive Pb from being transported into the protoplast (Wu et al., 2016), and removal of Cr from hydroponic solution in hybrid willows without showing detectable phytotoxicity (Yu and Gu, 2007). Lead can also be transferred to the vacuole, so as to reduce its concentration in protoplasts. Organic acids and proteins can precipitate Pb by chelation thereby reducing its free state. *Mirabilis jalapa* (The Marvel of Peru) demonstrates tolerance, rapid growth, and characteristics of native plants found in China, which is an ideal germplasm resource to restore soils contaminated by heavy metals (Zhou et al., 2012).

When plants are stressed by heavy metal contamination a change in growth, development and characteristics of accumulation occurs (Liang et al., 2011; Xu et al., 2012). In this paper, the enrichment concentration and tolerance of lead were investigated on hydroponically grown *M. jalapa.* Fourier transform infrared spectroscopy was used to determine chemical changes in different tissues and organs of *M. jalapa* when it was stressed by lead uptake (Bosch et al., 2006; Gu et al., 2009; Ohno et al., 2015). The objectives of the present study were to interpret *M. jalapa*'s physiological response to Pb toxicity and determine if it could tolerate high concentrations of Pb.

2. Materials and methods

2.1. Experiment method

M. jalapa seeds, collected from the tailings wasteland at the Xiangtan manganese mine, were sprinkled on sand-filled pots. Following germination (14 days), *M. jalapa* seedlings of the same size were selected, the roots were gently washed with tap-water and then thoroughly rinsed with deionized water. Plants were then grown in black plastic containers supplied with 20 L of Hoagland nutrient solution and exposed to 6 concentrations of Pb: 0 (control), 50, 100, 200, 500 and 1000 μmol L^{-1}, added as $Pb(NO_3)_2$(AR). Treatments in a glass house were replicated three times. All solutions were continuously aerated and pH maintained at 4.5 using 0.1 M NaOH or 0.1 M HCl, to ensure that Pb remained stable as Pb^{2+} in an ionic state. Plants were harvested after 35 days growth. Plant roots were then gently washed with tap water and then subsequently washed with deionized water and finally blotted dry with tissue paper. Plant samples were divided into roots, stems and leaves, which were firstly oven dried at 105 °C for 30 min and then at 75 °C until a constant weight was achieved (48 h). Fresh weight (hereafter referred to as FW) and dry weight (hereafter referred to as DW) of roots, stems and leaves were recorded. Samples of dry plant material for Pb analysis were ground to a fine powder using a stainless steel grinder, so as to pass through a 200 screen mesh.

2.2. Pb content analysis

Subsamples of dried plant tissue (c. 0.15 g) were digested with 90 mL of concentrated HNO_3 (AR, mass fraction = 65%), 30 mL of HCl (AR, mass fraction = 36–38%) and 3 mL of $HClO_4$ (AR, mass fraction = 70–72%) in a block heater. Lead was determined using ICP-OES (Jones et al., 2011).

2.3. FTIR analysis

The spectral information of various tissues and organs was characterized using Fourier transform infrared (FTIR) spectroscopy in the mid-IR range with a Nicolet IS10 infrared spectrometer. The detector was deuterated triglycine sulphate (DTGS). The measured wavebands ranged from 4000 to 500 cm^{-1} with a resolution of 1 cm^{-1}. Plant samples were finely ground with KBr (0.5/50 mg) using an agate pestle and mortar. Each sample was thoroughly mixed with sodium bromide particles and tableted into three almost transparent wafers. The samples were scanned 32 times and the three replicate spectra averaged to account for within sample variability and differences in particle size and packing density.

2.4. Statistical analysis

All analyses were performed in quintuplicate. The data were statistically treated with Microsoft Excel 2003, SPSS version 19.0 and Origin 8.0. In the case of homogeneity, Duncans post hoc test was used. If there was no homogeneity, Dunnetts T3 test was performed. All figures were constructed using Origin 8.0.

3. Results and discussion

3.1. Growth response of M. jalapa to Pb concentrations

M. jalapa grew normally under the range of Pb concentrations. However, at high concentrations (1000 μmol L^{-1}), the leaves rolled up slightly and the roots became black. Nevertheless, the different concentrations of Pb did not affect biomass production (Fig. 1). Therefore, a number of concentrations of Pb played a small role in *M. jalapa* growth and *M. jalapa* has a strong Pb tolerance ability in the growth medium.

3.2. Pb uptake and accumulation characteristics of M. jalapa

With an increase in Pb concentration, Pb accumulation increased in all parts of the plant (Table 1). The content of Pb was found in the order roots > leaves > stems. Even with different Pb concentrations, the content of Pb in stems was the same as that in the control ($P > 0.05$). However, Pb increased significantly when plants were exposed to high concentrations of 500 and 1000 μmol L^{-1} ($P < 0.05$) (see Table 2).

Translocation factor (Hereafter referred to as TF) reflects the transportation and distribution of metals in plants from below ground to above. A number of studies (Du et al., 2013; Tabaraki et al., 2014; Yin et al., 2011) have shown that Pb mostly accumulates in the roots, which therefore reduces transportation to the above ground tissues, which is one of the important mechanisms to reduce toxicity and has been demonstrated in this study. No clear trend was observed in the TF of *M. jalapa* stem and leaf tissues.

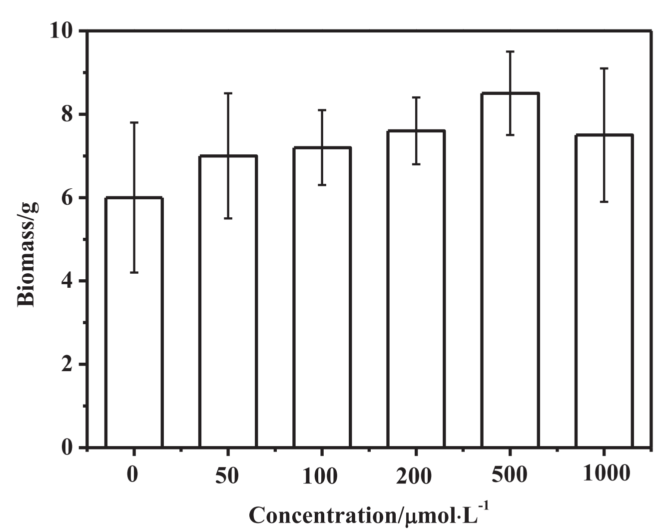

Fig. 1. Effects of various concentrations of Pb on the biomass of *M. jalapa.*