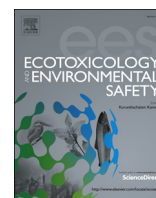




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## Cadmium uptake in above-ground parts of lettuce (*Lactuca sativa* L.)

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### ABSTRACT

Because of its high Cd uptake and translocation, lettuce is often used in Cd contamination studies. However, there is a lack of information on Cd accumulation in the above-ground parts of lettuce during the entire growing season. In this study, a field experiment was carried out in a Cd-contaminated area. Above-ground lettuce parts were sampled, and the Cd content was measured using a flame atomic absorption spectrophotometer (AAS). The results showed that the Cd concentration in the above-ground parts of lettuce increased from 2.70 to 3.62 mg kg<sup>-1</sup> during the seedling stage, but decreased from 3.62 to 2.40 mg kg<sup>-1</sup> during organogenesis and from 2.40 to 1.64 mg kg<sup>-1</sup> during bolting. The mean Cd concentration during the seedling stage was significantly higher than that during organogenesis ( $\alpha=0.05$ ) and bolting ( $\alpha=0.01$ ). The Cd accumulation in the above-ground parts of an individual lettuce plant could be described by a sigmoidal curve. Cadmium uptake during organogenesis was highest (80% of the total), whereas that during bolting was only 4.34%. This research further reveals that for Rome lettuce: (1) the highest Cd content of above-ground parts occurred at the end of the seedling phase; (2) the best harvest time with respect to Cd phytoaccumulation is at the end of the organogenesis stage; and (3) the organogenesis stage is the most suitable time to enhance phytoaccumulation efficiency by adjusting the root:shoot ratio.

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### 1. Introduction

Since the ITAI–ITAI disease was reported in the late 1950s, people have been concerned about Cd toxicity to human health (Hagino and Kono, 1955). Cadmium can cause kidney tubular and bone damage (Jarup and Akesson, 2009), and it is also involved in lung edema, liver damage, anemia, and hypertension (Baldantoni et al., 2016 and references within). Diet is the main source of the Cd intake for non-smokers (Jarup and Akesson, 2009). The maximum limit for human intake is 70 µg d<sup>-1</sup> (FAO-WHO, 1978), and the average cadmium intake from food generally varies between 8 and 25 µg d<sup>-1</sup> (Jarup and Akesson, 2009 and references within). The indiscriminate disposal of domestic and industrial waste material and application of P fertilizer are major sources of Cd enrichment in soils (Noll, 2003; Bolan et al., 2014). Under natural conditions, heavy metals are mainly unavailable for plants (Tyler et al., 1989; Das et al., 1997). However anthropogenic heavy metals in soils are easily translocated into the food chain. In addition, unlike organic pollutants, heavy metals can exist in soil for a long time (Adriano et al., 2004). Therefore, it is necessary to clean up Cd contamination in soil.

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Lettuce, a widely consumed vegetable, is very sensitive to the presence of toxic elements in soil, and these elements can accumulate in the edible parts of the plant (Do Nascimento Da Silva et al., 2015). Zorrig et al. (2013) reported that lettuce grown in the presence of 15.0 µM CdCl<sub>2</sub> had leaf Cd concentrations that were 100-fold higher than the legal maximum level for vegetable products marketed for human consumption. Both Zorrig et al. (2013) and Pereira et al. (2011) observed that no symptoms of dehydration, chlorosis, or necrosis were found on the investigated lettuce plants. Lettuce has even been considered as a Cd bio-indicator (Monteiro et al., 2009). Lettuce is good model plant for phytoaccumulation investigations, and it can be used to identify Cd uptake and its subsequent accumulation in edible plant tissue (Rashid et al., 2014). Therefore, lettuce is popular as an experimental plant for Cd studies (Podar and Ramsey, 2005; Kukier et al., 2010; Melo et al., 2012; Mehmood et al., 2013; Fontes et al., 2014; Do Nascimento Da Silva et al., 2015). In addition, the total global commercial production of lettuce reached up to 23.6 million metric tons in 2010 (Liu et al., 2014). The great commercial value of lettuce makes it necessary to protect it from Cd contamination.

In this study, we studied variation in Cd accumulation by the above-ground parts of Rome lettuce during the entire growth stage in field soil that had received phosphate application with excessive Cd for over 20 years. The purpose of the study was to

clarify during which stages Cd content and accumulation is highest. The most sensitive stages of lettuce growth were identified as bio-indicators of Cd contamination, and lettuce harvested during stages with the least contamination can be used for phytoremediation.

## 2. Methods and materials

### 2.1. Field and plant material

This experiment was carried out in a greenhouse located in Yangling, Shaanxi Province (N34°17'28", E108°00'27") during May 20–August 10, 2014. Soil in the greenhouse had previously received phosphate application for over 20 years. The soil was classified as loess soil, and some of the physical and chemical properties are listed in Table 1. The experimental greenhouse was located in a semi-humid area with an average annual precipitation of 595.9–732.9 mm, an average annual temperature of 13.6 °C, and total annual radiation of 440–544 kJ cm<sup>-2</sup> (Ji et al., 2015). One variety of Rome lettuce (*Lactuca sativa* L.) named “Li sheng er hao” was selected as the experimental species because of its high

biomass (300–500 g/plant) and suitable growing period (about 70 days from sowing to harvest).

### 2.2. Experimental layout

#### 2.2.1. Lettuce cultivation

Lettuce seeds were densely and uniformly cultivated in nine lines in the middle of the greenhouse after the surface soil was well mixed with a machine. Each line, with a length of 6 m and a width of 30 cm, was connected to the others. The cultivation area was covered with polyethylene plastic film to maintain moisture before germination. When necessary, water was sprayed to maintain 60% of the soil water holding capacity. No fertilizer was applied before or during the growth period.

#### 2.2.2. Sampling

Samples of above-ground lettuce parts were collected beginning on day 20 after sowing. The above-ground lettuce parts from 20 plants in the center of the cultivation area were collected on days 20, 25, 27, 30, 32, 33, 34, 35, 36, 38, 40, 42, 44, 46, 48, 51, 53, 57, 59, 61, 67, 70, and 73 after sowing. On the day of sampling, all the samples were pretreated with the same method for later analyses.

### 2.3. Sampling pretreatment and metal analysis

#### 2.3.1. Sampling pretreatment

Fresh lettuce samples collected from the field were carefully cleaned using distilled, high-purity water to remove surface dirt. Then, the samples were dried at 80 °C until a constant weight was reached. The dried samples were then crushed and uniformly mixed using a stainless steel plant tissue grinder (LD-Y500A, Shanghai, China). The fresh and dry weights of each sample were determined.

#### 2.3.2. Metal analysis

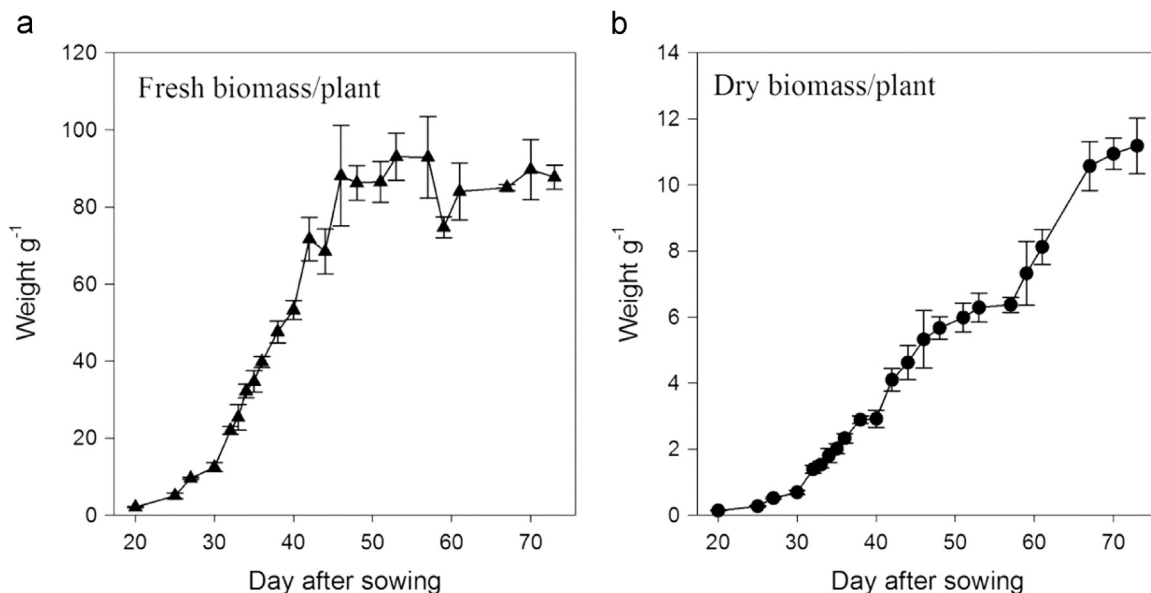
One-half gram of dry crushed sample was added into a quartz container, mixed with 9 ml of HNO<sub>3</sub> (GR) and 3 ml of HClO<sub>4</sub> (GR), and then digested at 160 °C until almost dry (Yan et al., 2015). The digested liquid was brought to a volume of 25 ml with high-purity water. The Cd content of the liquid samples was detected with a

**Table 1**

Properties of the 0–20 cm soil layer in the experimental field. Data shown are mean values ( $n=3$ ).

Parameter	Units	Value
Soil type		Heavy loam
pH (water: soil=5:1)		7.83 ± 0.1
Soil organic matter	g kg <sup>-1</sup>	36.12 ± 1.2
Total nitrogen	g kg <sup>-1</sup>	1.72 ± 0.06
Olsen phosphate	mg kg <sup>-1</sup>	302.6 ± 8.52
Available potassium	mg kg <sup>-1</sup>	721.6 ± 14.39
Cd	mg kg <sup>-1</sup>	1.87 ± 0.07
Available Cd <sup>a</sup>	mg kg <sup>-1</sup>	0.36 ± 0.03
Fe	%	3.3 ± 0.20
Cu	mg kg <sup>-1</sup>	25.9 ± 2.26
Zn	mg kg <sup>-1</sup>	73 ± 3.46
Ca	%	5.20 ± 0.17
Mg	%	1.34 ± 0.017

<sup>a</sup> Extracted for 2 h at 25 °C with DTPA (DTPA-TEA-CaCl<sub>2</sub>, pH7.3) buffer solution.



**Fig. 1.** Above-ground fresh biomass weight plant<sup>-1</sup> (a) and dry biomass weight plant<sup>-1</sup> (b) during the experimental period (day 20–73 after sowing). Day 20–30 was the seedling stage, 30–57 was the organogenesis stage, and 57–73 was the bolting stage. The error bars represent the stand errors of the means ( $n=3$  for each sample).

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