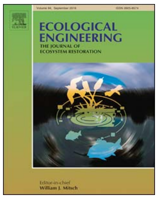
Ecological Engineering 116 (2018) 61–66

Contents lists available at ScienceDirect

Ecological Engineering

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



Medicago sativa L. enhances the phytoextraction of cadmium and zinc by *Ricinus communis* L. on contaminated land in situ

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ARTICLE INFO

Keywords:
Plant coexistence
Phytoextraction
Cadmium
Zinc
Ricinus communis L.
Medicago sativa L.

ABSTRACT

Crop co-planting is widely used in agriculture because it can increase total crop yields through increased resource use efficiency, and phytoremediation is based on the contaminant remediation system in plants. This study focused on the phytoextractive effects of co-planting *Ricinus communis* and/or legumes in Cd- and Zn-contaminated soil. A Cd- and Zn-contaminated factory relocation site in Shanghai was selected for the experiment, and according to the results of a potential ecological risk assessment of heavy metals, the study area was divided into 3 levels of pollution: slight, moderate, and high. The results showed that the presence of *Medicago sativa* can significantly increase the height and biomass of *R. communis*, and there was a greater impact on the chlorophyll content of *R. communis* at higher pollution levels. Differences in pollution levels could significantly change the oil content of *R. communis* plants, but *M. sativa* can alleviate the impact of heavy metals. The presence of *M. sativa* increased the cumulative amount of cadmium and zinc in *R. communis* by 1.14 and 2.19 times, respectively. In short, co-planting *R. communis* and legumes remediated contaminated soil and may be one practical phytoremediation pathway for heavy metal-contaminated soil in the future.

1. Introduction

As the world has undergone industrial economic growth, heavy metals have gradually accumulated in agricultural soils. It is said that more than 16.1% of soils in China are contaminated, including 7.0% with cadmium (Cd) and 0.9% with zinc (Zn) (Chen et al., 2016). Therefore, the remediation of contaminated soil is necessary to save land resources and protect human health (Kramer, 2005; Vangronsveld et al., 2009). Phytoremediation is based on the contaminant remediation system in plants, and it has recently become an economically, environmentally and aesthetically accepted technology (Ahmadpour et al., 2012; Conventionally, 2011; Majid et al., 2012).

Ricinus communis L. is an oilseed crop in the Euphorbiaceae family (Wale and Assegie, 2015) that has a high biomass and a well-developed root system. China produces the second largest amount of *R. communis* in the world (Bauddh and Singh, 2015), and the crop is also metal tolerant, with a high tolerance to Cd concentrations (Shi and Cai, 2009). *R. communis* has a higher remediation efficiency than Indian mustard (*Brassica juncea* L.), which is considered a potential phytoremediator (Bauddh and Singh, 2012), and it has a high phytoremediation potential for cadmium- and zinc-contaminated soils (Wang et al., 2016). However, fast-growing and high biomass plants often contain low to moderate concentrations of heavy metals (McGrath et al., 2001). Thus, researchers have studied several approaches to improve the potential for heavy metal phytoextraction using *R. communis*. One idea was to apply metal chelators, but some chemical chelators are relatively expensive and present environmental risks (Wu et al., 2006). In addition, a low concentration of chemical chelators can reduce plant biomass (Chen and Cutright, 2001). Furthermore, plants can increase metal bioavailability by themselves; experiments have shown that some plant roots can produce H^+ and small-molecular-weight organic acids that can increase the amount of bioavailable metals (Duarte et al., 2007).

Crop co-planting has been widely used in agriculture in China for 2000 years (Zhang and Li, 2003), and legumes are often included in such systems because of their ability to fix N_2 (Karpenstein-Machan and Stuelpnagel, 2000; van Kessel and Hartley, 2000). In this study, we assumed that N-fixers can provide additional N to the main crop plant, which can produce substantial biomass and thus enhance the phytoextraction of cadmium and zinc from contaminated soil. We used

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https://doi.org/10.1016/j.ecoleng.2018.02.004
Received 4 August 2017; Received in revised form 31 January 2018; Accepted 3 February 2018

Table 1
Soil properties.

pH	7.62
Organic matter (mg kg^{-1})	18777
Total N (mg kg^{-1})	836
Total P (mg kg^{-1})	548
Total Cd (mg kg^{-1})	0.32–2.77
Total Zn (mg kg^{-1})	152–1427

Medicago sativa L. as the legume species to intercrop with *R. communis* because it is a metal-tolerant plant that can tolerate both zinc and cadmium (Desjardins et al., 2016; Kabir et al., 2016). We focused on the phytoextraction of cadmium and zinc from contaminated soil, and our main objectives were to investigate (i) whether co-planting with the legume affected the chlorophyll and oil contents of *R. communis*, (ii) whether co-planting with the legume affected the growth and biomass of the phytoextractor (*R. communis*), and (iii) whether co-planting with the legume enhanced the phytoextraction of cadmium and zinc by *R. communis*.

2. Materials and methods

2.1. Field experiment

The field experiment was implemented in a 200-m^2 area of a factory relocation site in Shanghai (31°11′42″ N; 121°32′35″ E); the properties of the soil are described in Table 1. The field experiment was divided into thirty-six plots with an area of 1.5 m × 3 m each. Surface soil (0–20 cm) was collected to determine the Zn and Cd concentrations in each plot; the heavy metal concentration range is presented in Table 1. Based on the heavy metal concentration in each block, the Hakanson potential ecological risk assessment was used. RI was introduced to assess the degree of ecological risk of heavy metals in the soil and was calculated using the following equation:

$$C_f^i = C_s^i / C_n^i$$

$$E_r^i = T_f^i \times C_f^i$$

$$RI = \sum_{i=1}^{n} E_r^i$$

where RI is the sum of the potential risk of individual heavy metals; Ei r is the potential risk of individual heavy metals; Ti f is the toxic-response factor for a given heavy metal; Ci f is the contamination coefficient; Ci s is the current concentration of heavy metals in the soil; and Ci n is the pre-industrial record of the heavy metal concentration in the soil.

According to the results of the potential ecological risk assessment of heavy metals, the area was divided into three different pollution levels (Hakanson, 1980): slight (RI ⟨1 5 0), moderate (150 ≤ RI ⟨3 0 0), and high (300 ≤ RI). The experiment employed a two-factorial design with three different pollution levels and two plant types, *R. communis* alone and *R. communis* co-planted with *M. sativa*. The planting layout is shown in Fig. 1, and the corresponding plants were seeded in each plot according to the schematic. The seeding date was May 24, and the number of plant seeds sown were 5 grains of *R. communis* and 20 grains of *M. sativa*. All the seeds were sourced from the Shanghai market.

2.2. Plant harvest analysis

Plants were harvested 120 days after sowing, and the height and stem circumference at 15 cm of *R. communis* were measured with a metric scale. Plants were divided into roots, stems, leaves and fruits, which were washed with deionized water for further analysis.

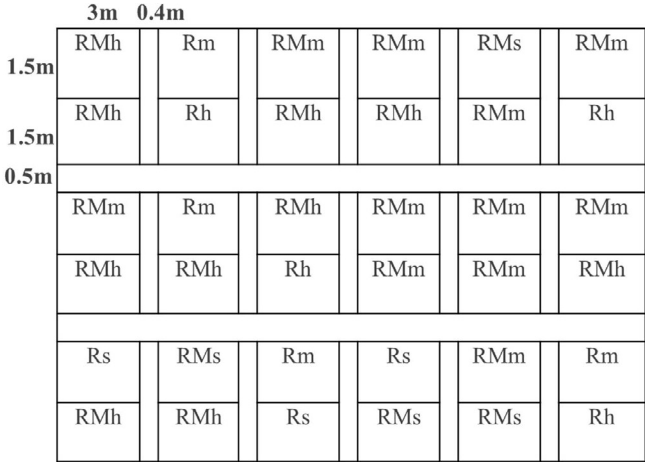

Fig. 1. The arrangement of *R. communis* and *M. sativa* experimental plats. Rs: *Ricinus communis* L. + Slight level of pollution, Rm: *Ricinus communis* L. + Moderate level of pollution, Rh: *Ricinus communis* L. + High level of pollution, RMs: *Ricinus communis* L. + *Medicago sativa* L. + Slight level of pollution, RMm: *Ricinus communis* L. + *Medicago sativa* L. + Moderate level of pollution, and RMh: *Ricinus communis* L. + *Medicago sativa* L. + High level of pollution.

2.3. Chlorophyll concentrations

Fresh leaf tissues (approximately 0.2 g) were cut in color-comparison tubes. Then, the samples were kept in 25 ml of 80% acetone and 95% ethanol (v:v = 2:1) for 24 h for complete extraction, and the total chlorophyll concentrations of *R. communis* were determined spectrophotometrically using the visible wavelength of 652 nm.

2.4. Oil content of fruits

The oil contents of fruits were determined by the Soxhlet extraction method. Fruits were dried in an oven at 70 °C for 48 h and then ground and passed through 0.25-mm sieves. Three-gram porphyrization samples were loaded into a weighed filter bag; the mouth of the package was sealed; and the samples were dried in a 105 + 2 °C oven for 3 h. The extraction barrel was placed with the sample, and petroleum ether was added. Samples were extracted for 12 h and kept at a water temperature of 70 °C. After extraction, the petroleum ether was volatilized in a ventilated area, and the samples were then dried in the 105 + 2 °C oven for 2 h and weighed.

2.5. Elemental analysis

Roots, stems, leaves and fruits were oven-dried at 105 °C for 30 min and then at 70 °C for 48 h. Dry biomass was powdered and digested (Liu et al., 2008), and the Cd and Zn concentrations were determined using ICP-AES (ICP, LEEMAN Company, United States) and AAS (ZEEnit600, Analytik Jena AG, Germany).

Soils were air-dried and passed through 0.25-mm sieves. The Cd and Zn concentrations from the soil samples were digested and analyzed by ICP-AES (ICP, LEEMAN Company, United States) and AAS (ZEEnit600, Analytik Jena AG, Germany).

2.6. Bioconcentration factor (BCF)

The bioconcentration factor (BCF) was used to evaluate the efficiency of Cd and Zn phytoextraction, and it was calculated using the following equation:

$$BCF = C_{plant}/C_{soil}$$

where C_{plant} (mg/kg) is the heavy metal concentration of the plant, including the roots, stems and leaves, and C_{soil} (mg/kg) is the heavy