Effects of root exudation on the accumulation of Cd by *Chenopodium ambrosioides* L. and maize in intercropping systems

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Abstract. Pot experiments were carried out to study the effects of intercropping by *Chenopodium ambrosioides* L. and maize on the root exudates of soluble sugar and amino acids, in order to understand the relationship between root exudates and cadmium accumulation in plants. The results showed that, compared with monoculture system: (1) after intercropping with maize, the content of soluble sugar in root exudates of *C. ambrosioides* increased by 106.54%, the content of amino acids increased by 52.73%, the available Cd content in rhizosphere soil increased by 12.09%, the root Cd content increased by 15.52%. (2) The content of soluble sugar in root exudates of maize increased by 40.41%, the content of amino acids increased by 70.59%, the available Cd content in rhizosphere soil increased by 29.35%, the shoot Cd content of maize increased by 208.26%. (3) There were significantly positive correlations between the contents of soluble sugar and amino acids secreted by *C. ambrosioides* and maize roots and the available Cd content in soil. In addition, there was a significant positive correlation between the available Cd content in soil and the Cd contents in the shoots and roots of plants. The results showed that the increase of soluble sugar and amino acid content of root exudates under the intercropping system caused the change of the available Cd content in the rhizosphere soil, which eventually changed the accumulation of Cd in the shoot and root of *C. ambrosioides* and maize.

1. Introduction

Phytoremediation is the best way to remediate Cd-contaminated soils. However, hyperaccumulator that is commonly used for phytoremediation often has low biomass and slow growth, leading to unsatisfactory effects [1]. Intercropping system to repair soil heavy metal pollution does not need to stop cultivating land for phytoremediation, to achieve edge production and control, with strong feasibility and development potential [2]. Intercropping hyperaccumulator with crops can facilitate metal uptake by the hyperaccumulator plants and concurrently reduce metal accumulation in the intercrops, which will ultimately improve the efficiency of the remediation and reduce the metal content of the crops [3-5].

About 50% of the intercropping advantage comes from the interaction between the underground parts of plants [6]. Dynamic changes in the rhizosphere environment of intercrops control the transformation, migration and fate of harmful substances in the plant–soil system. The interactive effect in the rhizosphere of two different plant species with intercropping results from the joint action of plants with specific microorganisms that are mediated by root exudates [7].
There are significant differences in the types and amount of root exudates between different plant species or different genotypes of the same species [8]. Soluble sugars and amino acids are the first substances discovered and isolated from plant root exudates, which have a great influence on rhizosphere process [9].

Maize is the main crop planted in the farmland surrounds the lead–zinc mining area in Huize, Yunnan Province, China. Chenopodium ambrosioides L. is a genus of amaranth, widely distributed in Yunnan, with rapid growth and large biomass. It has a high capacity for Cd uptake and transport [10]. In this paper, absorption and accumulation characteristics of Cd and changes of soluble sugar and amino acid content in roots were studied under the condition of C. ambrosioides intercropping with maize. The aim of the study was to demonstrate root exudation as a mechanism of Cd accumulation in the two plants and provide a reference for C. ambrosioides applying in the remediation of Cd-contaminated soils.

2. Materials and Methods

2.1. Experimental materials
C. ambrosioides seeds were collected from the Pb/Zn mining area of Huize County, Yunnan Province, and indoor seedlings were planted and potted experiments were carried out. Seeds of the maize cultivar Huidan No. 4 were purchased from a local seed company in Zhehai Town, Huize County. The tested soil was mixed with the slag soil of Yunnan Agricultural University and the lead-zinc ore slag in a ratio of 1:1. C. ambrosioides and maize seedlings were transplanted into the mixed soil for pot experiment. Physical and chemical properties of mixed soil: pH 7.89, organic matter contents of 57.12 g·kg−1, total N, P and K contents of 7.28, 6.9, 225.47 g·kg−1, respectively, available N, P and K contents of 160.9, 27.6, 460.7 mg·kg−1 respectively, and total Cd contents of 40 392 mg·kg−1.

2.2. Experimental design
The pot experiment was carried out in a test greenhouse. The pots had the dimensions of 40 ×10 × 20 cm and were filled with 5 kg of soil, and the soil sample is air-dried and passed through a 2 mm sieve. The three planting patterns included a C. ambrosioides monoculture, maize monoculture, and a C. ambrosioides/maize intercrop. When monocropped, two strains of seedlings were planted, and the plant spacing was 20 cm. When intercropped, one strain of C. ambrosioides and maize was kept, and the plant spacing was 20 cm. All the tests were carried out using the same water and fertilizer management measures throughout the growth of the plant. The plants were harvested 60 days later. Then, the plants with soil attached to the roots were brought back to the laboratory.

C. ambrosioides and maize plants were divided into underground (roots) and aboveground (stems and leaves) sections. All plant parts were washed with tap water and deionized water 3 times. The plant parts were placed in a drying oven at 105 °C for 30 min to deactivate the enzymes. After being air-dried, the samples were deactivated at 105°C for 30 min and then dried at 70°C to constant weight. Dry matter was weighed and the samples were subsequently pulverized, passed through a 0.25 mm screen, and stored in bags prior to analysis.

2.3. Collection of root exudates
Rinse the roots of the plants with tap water first, then rinse the roots with distilled water. During the cleaning of the root system, be careful not to damage the root system. At the same time, the intercropped schizonepeta and the roots of the maize are separated. The washed plant roots were immersed in 200 mL of 5 mg·L−1 thymol solution for 3 min, and the plant roots were placed in a collection bottle containing 200 mL of 0.005 mol·L−1 CaCl2 solution. Root exudates were collected by aeration for 6 hours under light conditions. The collected solution was concentrated to 10 mL by rotary evaporation at 50 °C, and then filtered through a 0.45 μm filter. Finally, the filtrate sample was stored in a refrigerator at -20 °C and stored for later use.
2.4. Sample Analysis
The available Cd content in the rhizosphere soil was extracted by DTPA (W:W = 1:2) and determined by atomic absorption spectrophotometry [8]. Plant Cd content was determined by digestion with HNO$_3$-HClO$_4$ (V:V=1:4) and determined by atomic absorption spectrophotometry. The content of soluble sugar secreted by roots was determined by anthrone colorimetric method, and the content of free amino acids was determined by ninhydrin colorimetry [9].

2.5. Data Processing
Data were analyzed using descriptive statistics in Excel 2007. Duncan’s multiple range test was used to determine significant differences. Correlation analysis was determined using the respective functions in SPSS 22.0. Significance thresholds were set at $P < 0.05$ (significant) and $P < 0.01$ (highly significant). Only significant relationships are reported.

3. Results and discussion

3.1. Effects of intercropping on plant root exudates
Root exudates are a general term for low molecular weight compounds (such as sugars, amino acids, and organic acids) secreted by the plant roots and released into the surrounding environment, and high molecular weight compounds (such as phytochelatins, plant high-iron carriers, metallo-sulphide-like proteins, etc.) [11]. Plant root exudates not only have a direct impact on the bioavailability of nutrients, but also have an indirect effect on plant roots and rhizosphere microorganisms. Plant rhizosphere microorganisms play a regulatory role in plant nutrient absorption [12]. Soluble sugar is the most important C source of plant rhizosphere microorganisms [13], and free amino acids are an important source of N source required for plant rhizosphere soil microbial growth [14]. In this experiment, intercropping altered the content of soluble sugar and free amino acid secreted by maize and C. ambrosioides roots (Fig. 1). Compared with the monoculture, the content of soluble sugar and free amino acid in the root exudates of maize and increased significantly, in intercropping. The soluble sugar content increased significantly by 106.54% and 40.41 ($P < 0.05$), the free amino acid increased significantly by 52.73% and 70.59% ($P < 0.05$), respectively. Intercropping of C. ambrosioides and maize increased the content of soluble sugar and free amino acids in plant root exudates, which provided an important material guarantee for increasing the number of microbes in rhizosphere soil. At the same time, the content of soluble sugar and free amino acids in plant root exudates were also changed due to the activities of rhizosphere soil microorganisms.

![Figure 1: Effects of intercropping on plant root exudations](image1)

**Figure 1** Effects of intercropping on plant root exudations (mg·L$^{-1}$). All values represent the mean ± standard error (SE), n=3, different lowercase letters mean significant difference ($P < 0.05$), the same below.

In fact, the intercropping influenced the roots exudation as reported by some studies [15,16]. Such as the intercropping of gramineous crops with peanuts (Arachis hypogaeae) resulted in the amount of organic acids and soluble sugars exuded by intercropped gramineous crops was higher than with
monocropped plants [15]. This study only analyzed the content of soluble sugar and free amino acids in plant root exudates, and did not deeply analyze the secretion rate of soluble sugar and free amino acids in plant root exudates and the changes of different components. These need to be further explored in future research.

3.2. Root exudation as a mechanism of intercropping for Cd accumulation
Intercropping can affect plant uptake and accumulation of heavy metals. For example, the intercropping of maize and peas reduces the copper content of the shoots and decreases the transport of copper from the roots to the shoots of maize [17]. The intercropping of T. caerulescens and barley improves Zn accumulation in the former and reduces Zn uptake by the latter [18]. Under Cd stress, tobacco (K326)–legume intercropping can significantly increase Cd uptake by the former [19]. In this study, the root Cd content of C. ambrosioides and the shoot Cd content of maize were significantly higher with intercropping than with monoculture (Fig. 2). Compared with monoculture, the Cd content in the roots of C. ambrosioides increased significantly by 15.52% (P<0.05), the shoots Cd content of maize increased by 208.26% (P<0.05). These results indicated that intercropping could promote Cd uptake by the hyperaccumulator C. ambrosioides and maize.

![Figure 2](image)

**Figure 2** Effects of intercropping on plant Cd content (mg·kg⁻¹)

The rhizosphere process of the plants will directly or indirectly affect the species transformation and bioavailability of heavy metals in the soil during intercropping and thereby affect the uptake, migration, transformation and accumulation of metals in the roots [20]. Intercropping is a cropping system that involves growing two or more crop species in close proximity. Due to plant interaction, the ecological processes of rhizosphere chemistry alter the rhizosphere environment in which the plants coexist, which affects the bioavailability of soil Cd under plant-coexisting conditions and further influences plant intake of Cd²⁺ ions. The rhizosphere soil is significantly influenced by the root exudates secreted into the rhizosphere [21]. And the effects of root exudates on the soils chemistry declined in the bulk soil. In this study, the available Cd content in the rhizosphere soil of C. ambrosioides and maize were significantly increased after intercropping (Fig. 3). The available Cd content in the rhizosphere soil of C. ambrosioides and maize increased by 12.09% and 29.35% (P<0.05) respectively, compared with monoculture. Therefore, the C. ambrosioides/maize intercrop resulted in an increase in the availability of Cd a in the soils.
Available Cd contents in rhizosphere (mg·kg\(^{-1}\))

| Plant Type          | Available Cd | Shoot Cd | Root Cd |
|---------------------|--------------|----------|---------|
| C. ambrosioides     | 0.826*       | 0.840*   | 0.965** |
| Maize               | 0.940**      | 0.986**  | 0.986** |
| C. ambrosioides     | 0.960**      | 0.989**  |         |
| Maize               | 0.935**      | 0.831*   |         |
| C. ambrosioides     | 1.000        | 0.957**  | 0.876*  |
| Maize               | 1.000        | 0.930**  | 0.888*  |

**"*" means significant difference (P < 0.05), "** means highly significant difference (P < 0.01)

Figure 3. Effects of intercropping on available Cd content in rhizosphere soil (mg·kg\(^{-1}\))

Correlation analyses were conducted between the soluble sugar and amino acid secreted by the plant roots and the available Cd contents in soils. Significant positive correlations were observed between the contents of amino acid and soluble sugar secreted by the C. ambrosioides and maize roots and the contents of available Cd in soils; their correlation coefficients were 0.826(P<0.05, n=6), 0.940(P<0.01, n=6), 0.960(P<0.01, n=6) and 0.935(P<0.01, n=6), respectively (Table 1). These results indicated that the soluble sugar and amino acid secreted by the roots of C. ambrosioides and maize had obvious effects on increasing the availability of Cd in the soils. At the same time, there were significant positive correlations between the shoot and root Cd contents of the and the contents of available Cd in soils, their correlation coefficients were 0.957(P<0.01, n=6), 0.876(P<0.05, n=6), 0.930(P<0.01, n=6) and 0.888(P<0.05, n=6), respectively. Hence, the functional mechanism of intercropping and its influence on the accumulation of Cd in plants was related to the roots exudations and their effects on the bioavailability of Cd in soil. Furthermore, the available Cd contents in soil were positively correlated with the shoots and roots Cd contents of C. ambrosioides and maize. These results indicated that the soluble sugar and amino acid in root exudates of C. ambrosioides and maize can facilitate the transformation of soil Cd to the available form, which increases the amount of Cd uptake and accumulation in plant.

Table 1 Correlation of root exudations and available Cd content in rhizosphere soil and plant Cd contents, plant Cd contents and available Cd content in soil (n=6)

| Plant Type          | Available Cd | Shoot Cd | Root Cd |
|---------------------|--------------|----------|---------|
| C. ambrosioides     | 0.826*       | 0.840*   | 0.965** |
| Maize               | 0.940**      | 0.986**  | 0.986** |
| C. ambrosioides     | 0.960**      | 0.989**  |         |
| Maize               | 0.935**      | 0.831*   |         |
| C. ambrosioides     | 1.000        | 0.957**  | 0.876*  |
| Maize               | 1.000        | 0.930**  | 0.888*  |

**"*" means significant difference (P < 0.05), **"** means highly significant difference (P < 0.01)

Summing up the correlations of the root exudations, available Cd contents in the soil, and the Cd contents in plants, observations indicated that intercropping increased the Cd contents in the shoots and roots of C. ambrosioides and maize, which was closely related to the effects of increasing the amount of exudations by plant roots on the availability of Cd in soil.

4. Conclusion
Intercropping of the Cd accumulator C. ambrosioides/maize resulted in an increase of Cd in the plants. Intercropping provided a new feasible way for enhancing the remediation efficiency of accumulators...
on polluted farmlands. Intercropping resulted in an increase soluble sugar and amino acid secreted by roots, and a increase in the contents of available Cd in soils was observed. Compared with monoculture, the content of Cd in the shoots of maize and in the roots of C. ambrosioides increased significantly under intercropping.

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