Effects of Intercropping on the Chemical Forms of Pb and Cd in Cyperus glomeralus and Zea mays.

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Abstract. Pot experiment was carried out to study the chemical form of Pb and Cd in two plants under the condition of Cyperus glomeratus and Zea mays intercropping. The results showed that: (1) The chemical form of Pb in two plants are mainly F_NaCl and F_R. Compared with monoculture, Pb content of various chemical forms in C. glomeratus increased by 5.88%-300% under intercropping, and decreased by 5.69%-47.69% in Z. mays. (2) The chemical form of Cd in both plants are F_NaCl and F_HCl, the F_R Cd contents in the shoot of C. glomeratus increased by 21.45% and the F_W, F_HCl and F_R Cd contents increased by 12.06%-33.33% under intercropping system; the F_HCl Cd contents in the shoot of Z. mays decreased by 21.84% and the F_W, F_NaCl, F_HAc and F_R Cd contents in the root of Z. mays decreased by 12.96%-61.03% under intercropping system comparing with monoculture system. It is shown that intercropping of C. glomeratus and Z. mays affects the chemical form of Cd and Pb in plants, promoting the absorption and accumulation of Pb and Cd in C. glomeratus, and inhibiting the accumulation of in Z. mays.

1. Introduction
With the development of mineral resources, large amounts of environmental pollutants were discharged into the natural environment and the soil was heavily polluted by heavy metals. Heavy metal pollution in the soil will seriously affect the growth of crops, yield and the quality of agricultural products, and toxic substances can even seriously endanger human health through the transmission of the food chain. Moreover, the incubation period of heavy metals is long, difficult to degrade, highly toxic, and cannot be repaired to the state before being polluted[1-2]. Phytoremediation has cheap economy, does not damage the soil structure, does not cause secondary pollution.However, the single use of hyper-accumulators to repair contaminated soil has the disadvantage of long cycle. The use of hyper-accumulators and low-accumulator crops may repair contaminated soil while harvesting qualified products[3]. It was found that the accumulation of heavy metals in crops could be effectively inhibited by promoting the absorption of heavy metals by enriched plants after intercropping with crops. Using the production technology found that the heavy metal polluted soil can be repaired even without agricultural production, which has great application value[4-6].

The heavy metal elements in the plant with oxalate, protein, phosphate, nitrate and other biochemical reactions will combine into various chemical forms, which is the mechanism of plant anti-heavy metal pollution elements. Heavy metals exist in various parts of plants in various chemical forms in plants[4], thus changing the migration and transformation of heavy metals in plants[5]. By using different extractants such as deionized water, ethanol, hydrochloric acid, acetic acid and sodium chloride, the heavy metal elements with different binding states can be extracted step by step by using the stepwise extraction method. The proportion of heavy metals in different plants is also very different. Previous
studies have shown that the content of NaCl, deionized water and ethanol of ultra-enriched plants is 86-96%, and with the increase of Cd concentration, the chemical forms of Cd will change from high activity to low activity\cite{7}. The acetic acid and hydrochloric acid extraction of Pb are dominant in wheat, rice, Z. mays and Zoysia japonica\cite{8,9}.

* C. glomeralus* is a widely growing perennial herb of the genus *C. glomeralus* in Yunnan, which has a significant enrichment of heavy metals. Previous studies have shown that the Pd, Cd elements in the soil can be heavily absorbed and transported by the papyrus\cite{10}. However, the mechanism of the system on heavy metal content of different chemical forms in *Z. mays* and *C. glomeralus* needs to be further studied. In this study, the contents and changes of Pd and Cd in *Z. mays* and *C. glomeralus* under intercropping system were studied, and the effects of intercropping on the chemical speciation distribution of Pb and Cd in plants were discussed, so as to provide reference for phytoremediation of heavy metal contaminated soil.

2. Materials and Methods

2.1 Test materials

*Z. mays* seeds ("Huidan 4") were selected from Huize County, *C. glomeralus* seeds were collected from Huize lead mining area in Yunnan. The soil tested was mixed with Pb-Zn mine residue in 1:1 ratio in Houshan red soil of Yunnan Agricultural University. Its basic physical and chemical properties are as follows: pH 7.69 and organic matter is 26.12 g·kg⁻¹, Full N 1.73 g·kg⁻¹, Full P 1.92 g·kg⁻¹, Full K 6.5 g·kg⁻¹, Alkalysis of N 56.61 mg·kg⁻¹, Speed-effect P 27.6 mg·kg⁻¹, Speed-effect K 211.9 mg·kg⁻¹, Total Pb 3427.2 mg·kg⁻¹, Total Cd 40.0 mg·kg⁻¹.

2.2 Experimental design

Pot experiments were carried out in three modes of *C. glomeralus/Z. mays* intercropping, *C. glomeralus* monoculture and *Z. mays* monoculture, with three parallel experiments in each mode. Sidried soil over 2mm and place in a foam basin 60 cm × 40 cm × 40 cm, 10 kg. per basin Single *C. glomeralus* and *Z. mays* left 3 seedlings, between the way is one *Z. mays*, two *C. glomeralus* Plants are harvested after 90 d.

2.3 Sample treatment and analysis

Divsededge and *Z. mays* into ground and underground, wash the sticky soil with tap water and steam.

Wash lush distilled water and dry naturally. Take 0.5000 g, with 80% alcohol (extract alcohol-soluble protein or amino acid salt F₁), Deionized water (extract water soluble organic acid and dihydrogen phosphate morphology FW.), 1 mol·L⁻¹.Sodium chloride (Extraction of pectin or absorbent heavy metal FNaCl.), 2% acetic acid (extraction of unsoluble heavy metal phosphate FHAc.), 0.6 mol·L⁻¹.Hydrochloric acid (extraction of oxalate binding state FHCl.), respectively. Transfer to 50 mL plastic centrifugal tube, \( 25^\circ C \) constant temperature oscillation after 22 h, 5000 r·min⁻¹.Centrifugal work at 10 min. Repeat the combined upper clearance twice. Residues after 5 extracts (F₅). Transfer with a small amount of deionized water to a triangular bottle, steamed on the electric heat plate, add 2 mL concentrated nitric acid and several drops of perchloric acid, boiled until clarification, lead, cadmium and zinc were determined by flame atomic absorption. The upper ant was determined directly by extractor. Soil effective state Pb, Cd, Zn content was extracted by DTPA (W:W = 1: 2) and measured by atomic absorption spectrophotometry\cite{11}.

2.4 Data analysis

A new complex pole difference method is adopted for the data difference significance analysis, routine using Microsoft Excel software and origin9.0. for mapping software.
3. Results and Analysis

3.1. Chemical forms of Cd in Z. mays

The extraction form distribution of upper Cd is: $F_{\text{HCl}}$(51.42%) > $F_{\text{W}}$(30.14%) > $F_{\text{NaCl}}$(8.69%) > $F_{\text{HAc}}$(6.20%) > $F_{R}$(3.55%); the extraction form distribution of the upper Cd in the intercropping Z. mays field is: $F_{\text{NaCl}}$(41.13%) > $F_{\text{HCl}}$(33.33%) > $F_{\text{W}}$(21.28%) > $F_{\text{HAc}}$(2.84%) > $F_{R}$(1.42%); compared to single, $F_{\text{NaCl}}$. The Cd content increased significantly by 373.3% (P <0.05), $F_{\text{HCl}}$ and the $F_{R}$. The Cd content was significantly decreased by 25.18% and 60.0% (P <0.05). The extraction morphological distribution of Cd from single Z. mays root is: $F_{\text{HCl}}$(38.10%) > $F_{\text{NaCl}}$(26.79%) > $F_{\text{W}}$(19.04%) > $F_{\text{HAc}}$(11.9%) > $F_{R}$(4.17%); the extraction form distribution of Cd after secondary Z. mays root is: $F_{\text{HCl}}$(48.28%) > $F_{\text{W}}$(23.28%) > $F_{\text{NaCl}}$(15.51%) > $F_{\text{HAc}}$(7.76%) > $F_{R}$(5.17%) The Cd content of the Z. mays roots was significantly reduced compared with that alone ($F_{R}$ except) (Figure 1).

3.2 Chemical forms of Cd in C. glomeratus

C. glomeratus above ground and roots FE. Cd was not detected during single and interval. The extraction form distribution of Cd is: $F_{\text{NaCl}}$(46.76%) > $F_{\text{HCl}}$(23.58%) > $F_{\text{W}}$(13.96%) > $F_{\text{HAc}}$(9.75%) > $F_{R}$(5.95%); the extraction morphological distribution of Cd is: $F_{\text{NaCl}}$(44.09%) > $F_{\text{HCl}}$(28.25%) > $F_{\text{W}}$(14.32%) > $F_{R}$(7.32%) > $F_{\text{HAc}}$(6.22%), compared to single, $F_{\text{HCl}}$. The Cd content was increased by 60%, and by $F_{\text{HAc}}$. The Cd content was significantly reduced by 54.19%. The extraction morphological distribution of Cd is: $F_{\text{NaCl}}$(39.15%) > $F_{\text{HCl}}$(32.62%) > $F_{\text{W}}$(17.94%) > $F_{R}$(5.72%) > $F_{\text{HAc}}$(4.57%) The extraction morphological distribution of C. glomeratus root Cd is: $F_{\text{NaCl}}$(42.93%) > $F_{\text{HCl}}$(32.01%) > $F_{\text{W}}$(17.33%) > $F_{R}$(5.06%) > $F_{\text{HAc}}$(2.67%), compared to single, C. glomeratus root $F_{\text{NaCl}}$, $F_{\text{HCl}}$. The Cd content was increased significantly by 42.11% and 26.71%. (Figure 2).
3.3 Chemical forms of Pb in Z. mays
The extraction form distribution of upper Pb is: $F_{NaCl}(34.74%) > F_R(22.8%) > F_{HAc}(16.46%) > F_{HCl}(12.69%) > F_E(11.41%) > F_W(9.1%)$; the extraction form distribution of the upper Pb in the intermediate Z. mays field is: $F_{NaCl}(27.07%) > F_R(25.22%) > F_E(18.9%) > F_{HAc}(16.16%) > F_{HCl}(8.89%) > F_W(3.69%)$, compared to single, Z. mays ground upper $F_E$, $F_W$. The Pb content has increased significantly by 25.17% and 59.12%.

3.4 Chemical forms of Pb in C. glomeratus
The extraction form distribution of Pb is: $F_{NaCl}(37.8%) > F_R(21.2%) > F_E(10.6%) > F_{HCl}(12%) > F_{HAc}(8.0%) > F_W(1.9%)$; the extraction form distribution of Pb is: $F_{NaCl}(37.9%) > F_R(19.4%) > F_E(14.2%) > F_{HCl}(13.4%) > F_{HAc}(7.6%) > F_W(7.4%)$, compared to single, C. glomeratus aboveground $F_E$ and the $F_W$. The Pb content increased significantly by 36.6% and 300%, compared with $F_{HAc}$ and the $F_R$. The Pb content of Pb was significantly reduced by 50.6% and 6.9%. The extraction forms distribution of C. glomeratus root Pb is: $F_{NaCl}(37.8%) > F_R(24.3%) > F_{HAc}(13.2%) > F_E(12.7%) > F_{HCl}(9.8%) > F_W(2.2%)$, compared with the single system, $F_R$, $F_W$, $F_{NaCl}$, $F_{HAc}$ and the $F_R$. The Pb content was all decreased significantly (Figure 4).
4. Discussion

Studies have shown that most heavy metal ions in plant cells exist in the form of chelates with polypeptides, amino acids, phosphate, organic acids, proteins and other organic compounds, which contain a large number of metal ion coordination groups, which is of great significance for maintaining the poisoning of heavy metals to plants\[11\].

Different chemical extraction of heavy metals in different plants. For example, the z. mays root leaves Pb mainly \( \text{F}_{\text{HAC}} \) and the \( \text{F}_{\text{HCl}} \), while \( \text{F}_{\text{W}} \). With the \( \text{F}_{\text{W}} \) minimum Pb content, Pb in golden grass and leaves Pb \( \text{F}_{\text{HCl}} \) mainly, followed by the acetic acid extraction state. In this experiment, Pb in Z. mays was mainly extracted from sodium chloride, indicating that Pb exists mainly in pectin, binding or absorbing state to protein. In comparison to the single work, After intercropping, The content of Pb in ethanol extraction state, deionized water extraction state, acetic acid extraction state and hydrochloric acid extraction state is significantly reduced, and the enrichment coefficient of Pb in Z. mays is also significantly reduced, after Intercropping with C. glomeratus, The content of Pb in Z. mays was significantly reduced, The cause of this phenomenon may be under the intermediary system, The roots of z. mays secrete organic acids, amino acids that combine with Pb, and change the PH value of Z. mays root system to be more suitable for transporting Pb, This brings more Pb, to the C. glomeratus side Given the strong enrichment effect of C. glomeratus on the Pb, This plays the detoxification role of Pb in Z. mays.

In this experiment, Cd mainly NaCl extraction and HCl extraction in C. glomeratus and Z. mays, indicating that Cd binds in plants with metal ligands such as polypeptide, oxalate and pectin, reducing the content of free Cd, significantly reducing the effectiveness and mobility of Cd, and Cd\(^{2+}\) Deposit on cell walls or transferring metal complexes from the cytoplasm to liquid bubbles is an important mechanism for plants with strong resistance to Cd. Plant tolerance to Cd has a significant positive correlation with the content of the NaCl extracted state Cd in barley root system is mainly HCl extraction state, and NaCl. Moreover, the sodium chloride extract state Cd accounted for more than 40% of the total Cd, respectively, indicating that the C. glomeratus has a strong tolerance to Cd.

Therefore, after working between C. glomeratus and z. mays, Cd, Pb can alleviate the heavy metal poisoning of z. mays to some extent.

5. Conclusion

(1) After intercropping, the Pb content of the grass ground and root increased, and the Pb content of the upper and root of the z. mays field was reduced. The chemical forms of Pb in both plants are dominated by less active sodium chloride extraction and residue states.

(2) After intercropping, the content of NaCl-extractable Cd in the upper part of z. mays increased significantly. The content of Cd in the roots of five extracts increased. The chemical forms of Cd in Z. mays and C. Glomeratus were mainly sodium chloride and hydrochloric acid.
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