Effects of Humus on Heavy Metals Absorption of Maize in Heavy Metal Contaminated Soil

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Abstract. To investigate the effect of humus (HS) on the growth of corn in heavy metal-contaminated soils, the present study first used sodium hydroxide (NaOH) to extract soluble humus (PHS) from peat soil and soluble humus from weathered lignite (LHS). Elemental analysis and Fourier transform infrared spectroscopy (FTIR) were used to analyze the chemical composition and functional group differences of the two HSs. Then, pot experiments were conducted to study the effects of LHS and PHS on soil carbon and nitrogen content, and on maize growth and the absorption of heavy metals in different plant parts. The main results showed that: (1) Compared with the control, corn biomass increased significantly when exogenously added 200mg/kg HS. The effect of HS on plant height and root length was different in both HS. The more hydrophilic PHS mainly affected the plant height of maize, while the more hydrophobic LHS mainly promoted the growth of maize root; (2) When PHS was applied at 100mg/kg, the Cd content of aerial parts of maize decreased significantly; when the application concentration of PHS was 200mg/kg, the content of Cu in the underground part of maize and the content of Cu and Zn in aerial parts decreased significantly; when the application concentration of PHS was 500mg/kg, the content of Zn in the underground part of maize was significantly reduced. When LHS was applied at a concentration of 200mg/kg, the content of Zn in the underground part of maize was significantly reduced. When LHS was applied at a concentration of 500mg/kg, the content of Cu in the underground part of maize and the content of Zn in the aerial parts were significantly reduced.

1. Introduction
Aiming at the characteristics of agricultural land pollution and without affecting its function as an agricultural production land, passivation technology that reduces the migration and bioavailability of heavy metals in soil by applying chemical amendments, in recent years, more and more attention has been paid to [1].

Humus is a kind of natural macromolecule organic matter produced by the decomposition and transformation of plants, animals and microbial residues, and plays an enormous role in improving the soil environment and maintaining soil function [2]. Studies have shown that exogenous applied humus can promote the interaction of various components in the soil through complex chemical actions and achieve the purpose of improving soil fertility [3]. Imbufe et al. [4] applied exogenously applied
potassium humus acid to effectively improve the agglomerate structure of acidic soils and salinized soils. In this study, two sources of humus were selected. Using indoor potting methods, different sources of humus were applied in heavy metal-contaminated soil and corn was planted. Changes in heavy metals in various parts of the corn were observed to investigate the effect of humus on the repair of heavy metal-contaminated soil.

2. Materials and Methods

2.1. Experimental design
The test soil was collected in a farmland soil near a tailings pond in Mouding County, Chuxiong Prefecture, Yunnan Province, and China. The specific GPS data is 25°36′75.26″N, 101°60′17.05″. After the test soil was naturally air-dried, it was crushed, ground, and sieved to 100 meshes and mixed. Accurately weigh 1.5 kg of soil into flower pots (internal diameter 20cm, height 15cm) and add weathering brown coal humus by treatment in blank control group (CK) and four concentrations (100, 200, 500, and 1000mg/kg). (LHS) and peat humus (PHS) and mix well with the soil, repeating each treatment three times. The tested corn seeds were disinfected with 10% hydrogen peroxide (H₂O₂) and washed repeatedly with distilled water, and then placed in the incubator for germination. After the seedlings were grown to 5cm or so, the seedlings with the same growing trend were selected, and 3 seeds were selected. The pots were transplanted into the test pots and harvested after 2 months and measured for the corresponding indicators. During the cultivation of plants, regular watering and weeding were performed, so that the soil moisture content of the test was maintained at 60% of the field water holding capacity.

2.2. Method of determination
Soil pH was measured by potentiometric method (soil-to-liquid ratio 1:2.5), and total nitrogen was determined by semi-micro Kjeldahl method. Soil TOC was determined using potassium dichromate-spectrophotometer method, the total amount of heavy metals and their bioavailable status in the soil were determined by aqua regia-HClO₄ digestion and diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) combined with atomic absorption spectrophotometer (Varian, AA240FS, USA). The basic physical and chemical properties of the tested soil were: pH 7.05, total nitrogen (TN) 1.25g/kg, total phosphorus 465.0mg/kg, total potassium 1.23g/kg, total soil organic carbon (TOC) 6.54g/kg, the total amounts of Cu and Zn in the soil exceeding the standard were 805.360mg/kg and 1053.708mg/kg, and the available Cu and Zn were 62.800mg/kg and 33.411mg/kg, respectively.

In this experiment, two kinds of peat soil and weathered lignite were selected as the source of humus extraction. The peat soil was bought from Klasman Company (Germany), and the weathered lignite was collected from Zhaotong, Yunnan. The method of extracting humus according to the method provided by Meng and other [5] is to sieve the peat soil and weathered lignite after 2 mm sieves, add 0.1 mol/L KOH in the proportion of 1:3 (m/v), vibrate 12 h at room temperature, retain the supernatant after low speed centrifugation, and then transfer the supernatant to pH 7 with 1 mol/L HCl, then transfer the solution. To dialysis bag, dialysis in distilled water environment until AgNO₃ cannot detect precipitation. Finally, the samples were freeze-dried and stored in a dryer for testing.

After the corn samples were harvested, the surface impurities were washed with tap water and ultrapure water, and then the water on the surface of the plants was dried. The fresh weight of the plants was measured, and the height and root length of the plants were measured. After soaking the plants with 1.5% hydrochloric acid for 15 minutes, wash the hydrochloric acid on the plant surface with tap water and ultrapure water, and fix it for 0.5 h at 105°C. Dry the sample to constant weight at 70°C, grind and mix with 5:1. Concentrate HNO₃-H₂O₂ (v/v) to digest the sample until the solution is clear and free of impurities, and then filter, after constant volume atomic absorption spectrometer (VarianAA240FS type) determination of Cu and Zn content.
2.3. **Data Analysis**

Experimental data were analyzed using SPSS 20 statistical software. One-way analysis of variance (One-way ANOVA) was used for significant difference analysis, and Tukey's method was used for multiple comparisons. The significant difference level was \( P<0.05 \), and the extremely significant level was \( P<0.01 \). Lab drawings use origin 9.0 software.

3. **Results and discussion**

3.1. **Analysis of Humus Elements**

Elemental analysis of humus is one of the basic methods for studying the structure and origin of humus. The elemental composition of LHS and PHS are arranged in descending order of atomic mass: carbon (C) > oxygen (O) > hydrogen (H) > sulfur (S) > nitrogen (N). PHS contains more C than LHS, while N and S have lower mass fractions than LHS. The C/N atomic ratio in PHS is higher than LHS, and the C/H atomic ratio is lower than LHS.

| sample | C (g/kg) | H (g/kg) | O (g/kg) | N (g/kg) | S (g/kg) | C/N ratio | C/H ratio |
|--------|----------|----------|----------|----------|----------|-----------|-----------|
| LHS    | 449.61±8.60 | 42.12±3.03 | 444.93±3.48 | 15.42±0.58 | 47.92±2.73 | 29.17     | 10.67     |
| PHS    | 465.34±3.65 | 52.53±1.04 | 452.30±3.68 | 12.39±0.10 | 17.44±1.11 | 37.55     | 8.86      |

3.2. **Humus FTIR Spectral Analysis**

FTIR is one of the main technical means for qualitative analysis of HS functional groups. The researchers used the peak position and peak intensity of the FTIR spectra to determine the functional groups contained in HS. The FTIR spectrum (Figure 3-1) shows that the two humus showed similar spectral images, but the local differences were significant. The main absorption peaks for LHS and PHS in this experiment are summarized in Table 3-2 [6,7]. In the 3700 ~ 2200 cm\(^{-1}\) region, the peak positions of the two peaks are similar, but the chemical shift of the LHS peak is slightly larger; the LHS shows a slightly stronger peak than the PHS in the 1800 ~ 1400 cm\(^{-1}\) region, among which the 1660 ~ 1630 cm\(^{-1}\) and The peak at 1450 cm\(^{-1}\) is stronger than that of PHS; the LHS has a stronger and denser absorption peak at 1350-400 cm\(^{-1}\).

![Figure 1. The FTIR spectra of Humus Substances](image-url)
Table 2. The assignment of absorption bands in FTIR spectra of HS.

| Band (cm⁻¹) | ascription                                                                 |
|------------|-----------------------------------------------------------------------------|
| 3700 ~ 2200| Phenol-OH, alcohol-OH or secondary amide NH                                  |
| 1660 ~ 1630| Aromatic ring C = C, C = O or ketone                                         |
| 1450       | Primary amides with C = N, or -COO-symmetric stretch                         |
| 1120       | Aliphatic C-OH or sulfone                                                   |
| 1080 ~ 1010| C-O stretching of polysaccharides or polysaccharides, silicate impurities of Si-O |
| 860-670    | Benzene ring = CH out of plane                                               |

In elemental analysis, according to Piccolo et al. [8], high N and S mass fractions indicate that LHS may contain more macromolecular structures of non-humus substances (polysaccharides or polypeptides); The higher C/H atomic ratio of LHS indicates that the degree of aromatization of LHS is higher, that is, LHS molecules have a higher degree of polymerization; Comparing the N content of humus acid in the paper of Puglisi et al. [9], the content of N in LHS and PHS was relatively low, but the C/N ratio of LHS and PHS was very high, indicating that both humus substances lack polypeptide derivatives.

In the FTIR spectra, the spectra of the two HS spectra are similar, but the peak intensity and the peak position are slightly different, indicating that the LHS and PHS contain similar functional groups, but the functional group content is different. In the range of 3700 ~ 2200 cm⁻¹, the peak of LHS is slightly stronger than PHS. According to the summary of Table 3-2, it can be considered that LHS may contain more hydroxyl (-OH); The peak positions at 1660 ~ 1630 cm⁻¹ and 1450 cm⁻¹ may indicate that the aromatic ring structure, C = N or -COO- bonds in LHS are more than PHS; Within the fingerprint region (1350 ~ 400 cm⁻¹) can be used to distinguish the slight differences in the structure of different compounds, HS in this region is a complex type of vibration and overlapping peaks, not easy to distinguish, however, strong LHS absorption peaks near 1000 cm⁻¹ can still be observed, which may indicate that the LHS structure contains more oxyalkyl groups.

3.3. Effect of humus on the absorption of heavy metals in different parts of corn

3.3.1. Effect of humus on Cu uptake in corn. As can be seen from Fig. 2, PHS and LHS of each application amount have an effect on the Cu content in corn, and the Cu content in the corn decreases with the increase of the HS application amount. In the PHS treatment group, when the application amount reached 200mg/kg, the Cu content in the underground and aboveground parts of the corn significantly decreased. When the PHS application amount was 200, 500 and 1000mg/kg, the Cu content in the underground part of the corn was respectively The percentages of Cu in the aboveground parts of maize decreased by 23.6%, 42.6% and 56.8%, respectively, decreased by 23.9%, 44.2% and 65.2%; in the LHS treated group, when the application amount was 500 and 1000mg/kg, the Cu content in the underground part of the plant appeared. The trend of significant decrease (compared with CK group) was a decrease of 28.9% and 41.8%, respectively. When LHS was applied at 200, 500 and 1000mg/kg, it effectively reduced the uptake of Cu in corn aboveground, with declines of 15.5%, 28.9% and 60.1%, respectively.
3.3.2. Effect of humus on Zn uptake by maize. Figure 3 shows the effect of exogenous application of humus on the absorption of Zn by various parts of corn. Exogenous application of 1000mg/kg PHS had the greatest effect on the Zn content in various parts of the plant. Under this treatment, the Zn content in the underground part of the maize decreased by 98.1%, and the part of Zn in the aerial part decreased by 99.1%, which was higher than the decrease of the LHS in the same application amount (98.1% and 97.2%, respectively). In addition, when the application amount was 500mg/kg, LHS had better control of the Zn content in the underground portion, which reduced the Zn content of the portion by 50.0%.

4. Conclusion
(1) Compared to peat humus, weathered lignite humus shows higher hydrophobicity and aromaticity.
(2) When the application concentration of PHS reached 100mg/kg, it had a significant effect on Cd uptake in maize shoots; when the application concentration of PHS reached 200mg/kg, there was a significant effect on Cu uptake in underground Cu and uptake of Cu and Zn in shoots; when PHS was applied at a concentration of 500mg/kg, the absorption of Zn in the subsurface of maize was significantly reduced. When LHS was applied at a concentration of 200mg/kg, the absorption of Zn in the subsurface of maize was significantly reduced. When LHS was applied at a concentration of 500mg/kg, the absorption of Cu in the underground part of the maize and the amount of Zn absorbed in the shoot were significantly reduced.
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