# Humus characteristics after maize residues degradation in soil amended with different copper concentrations

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**ABSTRACT**

Crop residues humification is an important process in nutrient cycling in agroecosystems. A preliminary laboratory incubation experiment of 180 days was conducted to evaluate the quantitative and qualitative characteristics of humic fractions formed during the humification process of maize residues in soil amended with different copper (Cu) concentrations. The Cu concentrations tested were 0, 50, 100, 200, 400, 600, 800, and 1000 mg Cu/kg dry soil. Results showed that the carbon content of humic fractions, i.e. humic acid (HA) and fulvic acid (FA), increased with the increase of Cu concentrations; the exception was carbon content of HA that initially decreased at the concentration of 50 mg Cu/kg. The color coefficient (ΔlogK) and activation degree (AD) of HA increased with increasing Cu concentrations while the relative color intensity (RF) decreased. In summary, we found that the carbon accumulation of humic fractions increases while the degrees of humification and aromaticity of HA decrease with increasing Cu concentrations in soil.

**Keywords**: humus composition; humic acid; color coefficient; relative color intensity; activation degree; maize residue; copper

Crop residues, the largest product of agricultural harvests, play a major role in nutrient cycling in agroecosystems by degradation. Two simultaneous processes are involved in degradation of crop residues, namely, mineralization and humification. Mineralization is the process through which the elements contained in organic form are converted to inorganic forms, while humification is the process where organic molecules are condensed into humus polymers (Bot and Benites 2005). Many factors, including temperature, moisture, pH, etc., can influence the two processes (Zech et al. 1997).

In recent years, inputs of heavy metals to soil ecosystems have increased as a consequence of anthropogenic activities. Many researches showed that heavy metals could influence the mineralization of crop residues by controlling the activity and composition of microbial community in soil (Giller et al. 1998, Khan 2000, Chander et al. 2002, Stemmer et al. 2007, Lahr et al. 2008). Because the roles played by humic substances in both nutrients cycling and heavy metals fate are important (Ghabbour and Davies 2003, Tan 2003, Perminova et al. 2005), it is essential to clarify the humification process of crop residues in soil. For the same reason as in the mineralization process, we can hypothesize that the inputs of heavy metals also affect the humification of crop residues in soil. However, to our knowledge, no comparable data are available on the humus characteristics after crop residues degradation in soil with different levels of heavy metal contamination.

Maize is one of the most important grain crops in the world. The production of maize returns large amounts of residues to the soil at harvest (Burgess et al. 2002). In addition, Cu is an important trace element; it is essential for life, but may

be toxic at high concentrations. Moreover, Cu has a high affinity for humic substances (Perminova et al. 2005). Thus, this preliminary study aims at assessment of the quantitative and qualitative characteristics of humic fractions formed during the humification process of maize residues in soil amended with different Cu concentrations under controlled incubation conditions.

## MATERIALS AND METHODS

**Incubation experiment design**. Soil sample was collected from the top 0–20 cm layer of a clay loam black soil (Argiudoll in USDA Soil Taxonomy) located at the Jilin Agricultural University, Jilin Province, Northeast China (43°48'51.6'' N, 125°24'42.1''E). The soil (< 2 mm) had a pH ($H_2O$) of 6.57 and contained 14.5 g/kg organic carbon and 20.6 mg/kg total Cu. The experimental maize residues (< 0.45 mm) contained 442.3 g/kg organic carbon and 16.2 mg/kg total Cu. The Cu solution used in the experiment was prepared from analytical grade cupric acetate, $Cu(CH_3COO)_2$.

At first, each soil sample of 100 g was pre-incubated for 24 h after the addition of different concentrations of Cu solution; the concentrations were 0, 50, 100, 200, 400, 800, and 1000 mg Cu/kg dry soil. The maize residue, to which $(NH_4)_2SO_4$ was added to give a carbon to nitrogen ratio of 10, was then mixed with the soil at a rate of 50 g/kg. All incubations were conducted at 30°C and 60% field moisture capacity. After 180 days of incubation, four replicates of each treatment were removed for the analysis.

**Analytical method**. The humus composition was analyzed according to the method described by Kumada (1987) with some modifications (Dou et al. 2007). By using the modified method, the water soluble and humic fractions could be quantified separately. Briefly, the soil samples were first suspended in distilled water at 70°C for 60 min, and then subsequently centrifuged. The supernatant was referred to as the water soluble fraction (WSF). The remaining soil was then extracted using a solution of 0.1 mol/l NaOH and 0.1 mol/l $Na_4P_2O_7$ at 70°C for 60 min. The dark brown alkaline supernatant solution, corresponding to the total alkali-soluble humic extract (HE), was separated into the acid-insoluble HA and the acid-soluble FA fractions by acidifying the alkaline supernatant to pH 1.0 with 0.5 mol/l $H_2SO_4$. HA was washed successively with 0.025 mol/l $H_2SO_4$ and water, and then dissolved in 0.05 mol/l NaOH.

The carbon contents of HE and HA were determined by $K_2Cr_2O_7$ oxidation method, while the FA carbon was calculated by subtracting the HA carbon from the HE carbon (Lao 1988). The color coefficient (Δlog K) and relative color intensity (RF) of the HA solution were obtained according to the following formulas:

$$\Delta\log K = \log K_{400} - \log K_{600}$$

Where: $K$ is the absorbance of HA solution at 400 or 600 nm.

$$RF = K_{600} (1000/V)$$

Where: $V$ is the number of milliliters of 0.02 mol/l $KMnO_4$ consumed by 30 ml of HA solution used in measuring the absorbance (Kumada 1987). The activation degree (AD) was measured using the $KMnO_4$ oxidation method (Dou et al. 2008). Treatment effects were analyzed using the one-way ANOVA, followed by LSD multiple comparison tests at $P < 0.05$ (SPSS 16.0).

## RESULT AND DISCUSSION

**Humus composition of soil**. The total soil organic carbon content was found to increase gradually from 15.8 to 20.9 g/kg with increasing Cu concentrations from 0 to 1000 mg/kg (Table 1). This indicates that there is a decreased mineralization of total soil organic carbon. The calculated carbon mineralization rate decreased from 56.8% for 0 mg Cu/kg to 42.9% for 1000 mg Cu/kg (data not shown). This is in agreement with previous results that show that heavy metals generally inhibit the mineralization of organic carbon by changing the activity and community composition of microbial decomposers in soil (Giller et al. 1998, Khan 2000, Chander et al. 2002, Clemente et al. 2006, Stemmer et al. 2007, Lahr et al. 2008).

With increasing Cu concentrations, the carbon content of WSF first decreased and then increased. On the other hand, the carbon content of the humic fractions gradually increased, given the same increase in Cu concentrations. The exception was that for the Cu concentration of 50 mg Cu/kg, where the carbon content HA decreased from 3.21 g/kg for 0 mg Cu/kg to 3.05 g/kg for 50 mg Cu/kg (Table 1). But the carbon contents of HA from 100 to 1000 mg Cu/kg were all higher than that of HA from 0 to 50 mg Cu/kg. This indicates that there was a gradual carbon accumulation for the humic fractions. It has been known that humic substances could bind to clay minerals through cationic bridges (Arnarson et al. 2000, Perminova et al. 2005). Therefore, one

Table 1. Effect of different Cu concentrations on humus composition of soil after maize residues degradation

| Cu added (mg/kg dry soil) | TOC[a] | WSF[b] | HE[c] | HA[d] | FA[e] | PQ[f] (%) |
|---|---|---|---|---|---|---|
| | | | (g C/kg soil) | | | |
| 0 | $15.8 \pm 0.8^e$ | $0.079 \pm 0.003^c$ | $5.17 \pm 0.10^e$ | $3.21 \pm 0.19^d$ | $1.96 \pm 0.16^c$ | $62.1 \pm 3.1^a$ |
| 50 | $16.3 \pm 0.4^{de}$ | $0.067 \pm 0.006^d$ | $5.47 \pm 0.06^d$ | $3.05 \pm 0.06^e$ | $2.42 \pm 0.10^b$ | $55.7 \pm 1.5^c$ |
| 100 | $16.7 \pm 1.3^{de}$ | $0.056 \pm 0.005^e$ | $5.76 \pm 0.07^c$ | $3.35 \pm 0.05^c$ | $2.41 \pm 0.07^b$ | $58.2 \pm 1.0^b$ |
| 200 | $17.0 \pm 0.6^d$ | $0.056 \pm 0.003^e$ | $5.82 \pm 0.03^c$ | $3.39 \pm 0.09^c$ | $2.43 \pm 0.11^b$ | $58.2 \pm 1.8^b$ |
| 400 | $18.5 \pm 0.5^c$ | $0.057 \pm 0.003^e$ | $6.02 \pm 0.19^b$ | $3.59 \pm 0.07^b$ | $2.43 \pm 0.13^b$ | $59.6 \pm 1.0^b$ |
| 600 | $19.6 \pm 0.6^{bc}$ | $0.096 \pm 0.008^{ab}$ | $6.13 \pm 0.02^b$ | $3.64 \pm 0.04^{ab}$ | $2.49 \pm 0.05^b$ | $59.3 \pm 0.7^b$ |
| 800 | $20.5 \pm 0.7^{ab}$ | $0.089 \pm 0.007^b$ | $6.29 \pm 0.09^a$ | $3.73 \pm 0.08^a$ | $2.56 \pm 0.04^{ab}$ | $59.3 \pm 0.6^b$ |
| 1000 | $20.9 \pm 0.6^a$ | $0.101 \pm 0.004^a$ | $6.41 \pm 0.16^a$ | $3.74 \pm 0.08^a$ | $2.67 \pm 0.19^a$ | $58.4 \pm 2.0^b$ |

Mean value ± standard error of four replicates are presented. Values in a column followed by the same letter are not significantly ($P < 0.05$) different. [a]organic C content of total soil; [b]organic C content of water soluble fraction; [c]organic C content of total alkali-soluble humic extract; [d]organic C content of humic acid fraction; [e]organic C content of fulvic acid fraction; [f]ratio of the amount of humic acid to the summed amounts of humic and fulvic acids

potential explanation for this carbon accumulation in humic fractions may be that the fractions are more protected from microbial decomposition by the formation of stable organic-metal-mineral complexes in soil with high than with low Cu concentration; yet, the formation for these fractions was possibly higher due to higher microbial activity in soil with low Cu concentration. In accordance with our result, Russell and Alberti (1998) showed an accumulation of humic substances of up to 200% in the soil heavily contaminated with Pb, Cd, and Zn, relative to the reference soil in two beech forest sites in the vicinity of a mining area.

The precipitation ratio (PQ), which is the percentage amount of HA to HE, was significantly lower at all Cu concentrations above 50 mg Cu/kg than at Cu concentration of 0 mg Cu/kg (Table 1). This indicates that Cu addition led to a higher proportion of FA in the soil. On the other hand, PQ tended to increase with Cu concentrations between 50 and 1000 mg Cu/kg, suggesting that the relative proportion of HA increased with increasing Cu concentrations. There was a significant difference in PQ between 50 and 100 mg Cu/kg, but no significant difference between 100 and 1000 mg Cu/kg. It has been known that HA and FA play different roles in the mobility and bioavailability of metal ions (Gondar et al. 2006). Heavy metals bound to FA are relatively more mobile and bioavailable than those bound to HA. Our results thus imply that Cu is more mobile and bioavailable in the low Cu contaminated soil following the addition of maize residues, but this mobility and bioavailability declines when Cu concentrations increase.

**Humic acid properties**. The Δlog K and RF have been used as indexes for the degree of humification of HA (Kumada 1987). When Cu concentrations were increased from 0 to 1000 mg/kg, the Δlog K of HA increased from 0.615 to 0.632, while RF decreased from 65.2 to 41.6 (Table 2). This indicates that HA formed during humification process of maize residues has a lower humification degree in high Cu concentrations of soil. According to Kumada (1987), all HA was classified as HA of type B. In a previous study in the vicinity of a mining area, Russell and Alberti (1998) reported that humic substances were less humified or polymerized in the heavily contaminated soil than in the reference soil. This finding agrees with our result.

The AD could reflect the ratio of aliphatic to aromatic structures in HA molecules (Dou et al. 2008). In our study, the AD of HA increased with increasing Cu concentrations (Table 2), indicating that the aliphaticity increased or aromaticity decreased in HA molecules. The rich aliphatic group content is an important characterization of newly formed or young HA (Kononova and Alexandrova 1973). The increase of AD was in keeping with the increase of Δlog K and the decrease of RF value. Presumably, the aliphatic-rich characterization of HA molecules under Cu addition may be partly ascribed to the contribution of Cu-induced resistant microorganism.

Table 2. Effect of different Cu concentrations on humic acid properties of soil after maize residues degradation

| Cu added (mg/kg dry soil) | Δlog K[a] | RF[b] | AD[d] |
|---|---|---|---|
| 0 | 0.615 ± 0.002[b] | 65.2 ± 1.6[a] | 33.3 ± 1.0[f] |
| 50 | 0.622 ± 0.007[ab] | 60.8 ± 0.2[b] | 37.1 ± 0.6[de] |
| 100 | 0.622 ± 0.001[ab] | 58.4 ± 1.7[b] | 35.6 ± 0.8[ef] |
| 200 | 0.622 ± 0.005[ab] | 55.6 ± 0.6[c] | 39.5 ± 0.9[bcd] |
| 400 | 0.634 ± 0.021[a] | 54.4 ± 0.6[c] | 38.0 ± 1.5[cde] |
| 600 | 0.628 ± 0.008[ab] | 50.8 ± 3.4[d] | 40.1 ± 1.1[bc] |
| 800 | 0.633 ± 0.011[a] | 48.4 ± 2.9[d] | 42.1 ± 3.7[b] |
| 1000 | 0.632 ± 0.002[a] | 41.6 ± 2.1[e] | 51.0 ± 2.7[a] |

Mean value ± standard error of four replicates are presented. Values in a column followed by the same letter are not significantly ($P < 0.05$) different. [a]logarithm of the ratio of the absorbance of HA at 400 nm to that at 600 nm; [b]absorbance of HA at 600 nm multiplied by 1000, and then divided by the number of milliliters of 0.02M $KMnO_4$ consumed by 30 ml solution of HA; [c]classification diagram by Kumada (1987); [d]organic C of HA oxidized by the $KMnO_4$ divided by that oxidized by the $K_2Cr_2O_7$-$H_2SO_4$ solution

Our preliminary results demonstrate that the addition of different Cu concentrations influence the quantity and quality of humic fractions formed during the humification process of maize residues in soil. This finding is potentially useful for the study of carbon cycling and the remediation of Cu contaminated soil. Further studies are needed for quantifying organic carbon in humic fractions deriving from the maize residues by carbon isotopic ($^{14}$C or $^{13}$C) methods. In addition, the structural composition of humic fractions needs to be further characterized using other, more sensitive and accurate methods such as FTIR and $^{13}$C NMR.

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