Detoxifying Ability of the Phenol-Enriched and Cross-Linked Humic Derivatives with Respect to Copper

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1. INTRODUCTION

Humic substances (HS) are widely known to play a mitigating role in polluted terrestrial and aquatic environments. Copper is one of the most widely spread environmental pollutant. The main mechanism of the detoxifying ability of HS in relation to heavy metals in general and copper in particular is the formation of non-bioavailable complexes. Hence, chemical modification of HS can be a promising tool to enhance the detoxifying capacity of HS. In particular an enrichment of the structure of HS with complexing fragments can be aimed.
The objective of this study was to estimate the detoxifying ability of humic derivatives which are artificially enriched in phenol, hydroquinone and catechol moieties with respect to copper(II).

2. MATERIALS AND METHODS

Commercially available potassium humate of humic acids of leonardite (CHP) produced by Humintech Ltd, Germany was used for modifications. Five preparations of modified HS were obtained:

- preparation CHP-PH-N250: CHP copolymerized with phenol by formaldehyde condensation, ratio: 1000 mg CHP/250 mg phenol;
- preparation CHP-PH-PC250A: CHP copolymerized with phenol and catechol by formaldehyde condensation, ratio CHP/phenol/catechol 1000/125/125 mg and cross-linked by hexamethylenetetramine;

- preparation CHP-PH-HQ250A: CHP copolymerized with phenol and hydroquinone by formaldehyde condensation, ratio HP/phenol/hydroquinone 1000/125/125 mg and cross-linked by hexamethylene-tetramine;
- preparation CHP-PH-PC250P: CHP copolymerized with phenol and catechol by formaldehyde condensation, ratio CHP/phenol/catechol 1000/125/125 mg and cross-linked by paraformaldehyde.

The elemental composition and total acidity values obtained for the preparations are shown in Table 1.

Table 1: Elemental and functional group composition of the HS derivatives

Sample	Content of elements on ash-free basis, mass %							Total acidity, mmol/g
	C	H	N	O	H/C	O/C	Ash	
CHP	60.7	4.70	1.40	33.3	0.93	0.41	12	4.8±0.3
CHP-PH-N250	61.9	4.12	1.09	32.9	0.80	0.40	8	5.9±0,4
CHP-PH-PC250A	58.1	4.19	2.73	35.0	0.86	0.45	5	5.6±0.6
CHP-PH-HQ250A	58.7	4.19	3.22	33.8	0.85	0.43	6	6.4±0.6
CHP-PH-PC250P	59.4	4.01	1.03	35.6	0.81	0.45	9	6.1±0.5

The total acidity of the humic derivates were higher than the parent HS (CHP). This fact reflects an increase quantity of phenolic groups in structure of modified preparations as a result of modification. The complexing ability of the humic dervivates increased due to changes in the HS structure. On the other hand, the elemental content of all preparations is typical for HS (1). Hence, the general structure of modified HS was not changed by the copolymerization.

The biological activity of the preparations was estimated by bioassay techniques using seedlings. The length of the longest root of seedlings of wheat *Triticum aestivum L.* was used as a test-response. Ten wheat seeds were placed in Petri dishes with solutions containing 10 mL of 5, 15, 30, 50, 100 mg/L of HS and 1 mg/L of Cu(II) in form of $CuSO_4$ if required.

The pH values of all solutions were adjusted to 5.5 - 5.9. Seeds were grown for 72 hours at 25 °C in the dark.

For quantitative assessment of the detoxification ability of the HS the detoxification coefficients (D) and the toxicological constants of copper binding

to HS normalized to the organic carbon content in HS preparations (K_{OC}^{tox}) were calculated as described in (2) according to the following equations:

$$D = \left(1 - \frac{R_d - R_{d+t}}{R_d} \Big/ \frac{R_o - R_t}{R_o}\right) \text{ and } D = \frac{K_{OC}^{tox} \times C_{HS}}{1 + K_{OC}^{tox} \times C_{HS}}$$

R_0 roots length of control

R_d roots length in presence of HS

R_t roots length in presence of copper

R_{d+t} roots length in presence of copper and HS

C_{HS} concentration of HS

The obtained values of K_{OC}^{tox} values were used for comparison of the detoxifying ability of HS preparations in relation to copper.

3. RESULTS AND DISCUSSION

The results on the detoxifying activity of the cross-linked humic copolymers are given in Figure 1.

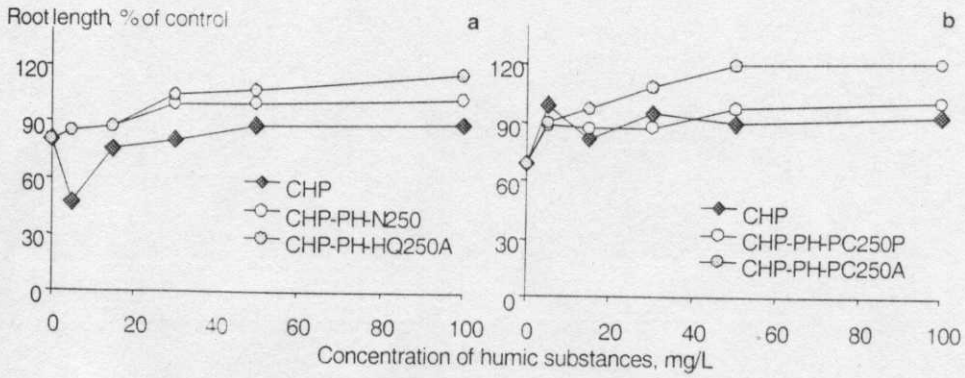

Figure 1: Dose-response relationships of parent HS and modified HS in the presence of 1 mg/L Cu^{2+}.

The toxicity of copper decreased significantly in the presence of the HS preparations (Fig. 1). At concentrations of 50 to 100 mg/L no copper toxicity was registered. In most cases at low HS concentrations (5-30 mg/L) the detoxification activity towards copper was higher for the humic derivatives than for the parent HS. The copolymers CHP-PH-PC250A showed the highest detoxifying ability. Hence, the detoxifying ability of the cross-linked derivatives

was higher compared to the parent HS and compared to the phenol-enriched derivatives.

The calculated values of the toxicological binding constants of the HS studied with copper(II) are given in Table 2.

Table 2: Toxicological constants of copper binding to CHP and its derivatives.

HS Cipher	CHP	CHP-PH-N250	CHP-PH-HQ250A	CHP-PH-PC-250P	CHP-PH-PC-250A
K_{OC}^{tox}, L/kg C	5.2×10^4	3.5×10^5	4.1×10^5	5.1×10^5	6.8×10^5

The data presented in Table 2 confirm the conclusion on the detoxifying ability of the modified HS as discussed above. K_{OC}^{tox} values of all modified HS were higher than the K_{OC}^{tox} value of the parent HS. Maximum value of K_{OC}^{tox} was observed for CHP-PH-PC-250A, a cross-linked derivative of catechol-enriched humic copolymer. The values of K_{OC}^{tox} of cross-linked derivatives exceeded that of non cross-linked copolymer. On the other hand, phenol copolymer showed higher detoxifying ability compared to the parent HS.

The HS investigated can be put into the following ascending order according to their detoxifying ability with respect to copper:

potassium humate of leonardite humic acid < phenol-enriched copolymer < crosslinked phenol-enriched copolymer < crosslinked catechol-enriched copolymer.

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REFERENCES

(1) Rice, J. A., MacCarthy, P.: Stastical evaluation of the elemental composition of humic substances. Org. Geochem 17 (5), 635-648 (1991).

(2) Perminova, I.V. et al.: Quantification and prediction of detoxifying properties of humic substances to polycyclic aromatic hydrocarbons related to chemical binding. Environ. Sci. Technol. 35, 3841-3848 (2001).