



Enhanced detoxifying ability of the specifically oxidized humic derivatives with respect to copper

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Introduction Copper is one of the most widely spread pollutant in the aquatic and soil environments where humic substances (HS) are an obligatory component. Due to the presence of carboxyl and phenolic groups in the structure of HS, they possess high affinity for complexing heavy metals, in general, and of copper, in particular (Lorenzo et al., 2002, Schnitzer and Khan, 1972). Binding with humics causes a reduction in the concentration of the most biologically active form of metals – in the freely dissolved species of metal cations. As a result, humics display detoxifying effects in the metal contaminated environments. To enhance detoxifying capacity of HS, chemical modification can be a promising tool, in particular, if it is aimed at enrichment of the structure of HS with hydroxyls. To validate this approach, the quantitative studies on detoxifying ability of the hydroxylated HA in relation to heavy metals are in need. The objective of this study was to estimate detoxifying ability of humic derivatives enriched with hydroxyls by means of oxidation technique with respect to copper (II).

Experimental Section *Humic materials used; methods of their oxidation and characterization.* The parent humic material was enriched with hydroxyl groups by means of conversion of phenolic moieties into hydroquinonic ones using specific oxidizing agents. Such modification was supposed to increase detoxifying ability of HS toward copper. Leonardite humic acids isolated from commercially available potassium humate Powhumus (Humintech, Germany) were used as a parent humic material (CHP). Three oxidizing agents were used: Elbs reagent (potassium persulfate); Fenton's reagent ($H_2O_2+Fe(II)$) and Fremy's salt (potassium nitrosodisulfonate). the

corresponding humic derivatives were designated as CHP-OEI, CHP-OFe, and CHP-OFr, respectively. The preparations used were characterized using elemental analysis, titrimetric analysis and ¹³C NMR-spectroscopy. The total and carboxylic acidity of the preparations was determined using standard barita and calcium acetate techniques, respectively (Swift, 1996). An amount of phenolic groups (Ar-OH) was calculated by subtracting carboxylic acidity from the total acidity value

Toxicity assays. Toxicity of copper was estimated using seedling technique. Wheat (*Triticum aestivum* L. cv. Moskovskaya-39) was used as a biotarget. The length of wheat roots was used as a test response. Wheat seeds were placed in water solutions containing 1 mg/L Cu²⁺ (solution CuSO₄) and 5, 15, 30, 50, or 100 mg/L HS. As controls, solutions of HS without Cu²⁺, solution of 1 mg/L Cu²⁺ and distilled water were used. pH 5.9 was maintained in all the experiments. Seeds were grown for 72 hours at 25°C.

Data treatment. For quantitative estimation of detoxification ability of the oxidized derivatives, the detoxification coefficients (D) were calculated as described in (Perminova et al., 2001). D was calculated as follows:

$$D = \left(1 - \frac{R_d - R_{d+t}}{R_d} \right) / \left(\frac{R_0 - R_t}{R_0} \right)$$

where: R₀ – 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.

On the basis of the D values, the toxicological constants of copper binding to HA normalized to the organic carbon content in HA preparation (K_{OC}^{tox}) were calculated as described previously (Perminova et al., 2001). The values of K_{OC}^{tox} were obtained by approximating the following expression:

$$D = \frac{K_{OC}^{tox} \times C_{HS}}{1 + K_{OC}^{tox} \times C_{HS}} \text{ where } C$$

C is a concentration of HS.

Results and Discussion To characterize the modification degree of the parent humic material as a result of its treatment with the selected oxidizing agents, the content of acidic groups was determined in all preparations used in this study (Table 1).

Table 1 – Content of acidic groups in the humic materials used in this study

Humic material	Total acidity (mmol/g)	COOH (mmol/g)	Ar-OH (mmol/g)
CHP	5.3	4.2	1.1
CHP-OEI	6.0	5.2	0.8
CHP-OFr	5.8	4.5	1.3
CHP-OFe	6.9	4.2	2.7

The total acidity of the oxidized preparations was higher than the corresponding parameter for the parent humic material. The carboxylic acidity varied in the range from 4.2 to 5.2 mmol/g. The content of phenolic groups was maximal in CHP-OFe, and minimal – in CHP-OEI.

Binding constants to copper determined from toxicological experiments (K_{OC}^{tox}) were used as quantitative characteristics of the detoxifying ability of the humic materials used in this study. The corresponding value for the parent humic material (CHP) was 52000 L/kg C. For all oxidized derivatives studied this parameter was much higher reaching its maximum value of 5580000 L/kg C for Fenton's oxidized derivative. Hence, the HS preparations under study can be put into the following ascending order according to their detoxifying ability with respect to copper: CHP < CHP-OEI << CHP-Fr << CHP-Fe. It should be noted that the preparation exhibiting the largest detoxifying ability with respect to copper was characterized with the highest total and phenolic acidity. This could be indicative of a leading role of phenolic hydroxyls in the complexation of copper by humic materials.

Conclusion

The hydroxyl-enriched humic derivatives obtained from leonardite humic acid using oxidation of phenolic fragments displayed higher detoxifying ability with respect to copper compared to the initial humic material. The most efficient oxidizing reagent was found to be Fenton reagent. Chemical modification is shown to be a promising tool for preparing humic detoxicants of the desired remedial properties.

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