Synthesis, Biological Activity and Detoxifying Properties of Carbonylated Humic Substances

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1. INTRODUCTION

It is known that functionally enriched humic substances possess high binding ability in respect to heavy metals (1). The promising approach for enhancing detoxifying ability of humic derivatives (HD) with respect to heavy metals is incorporation of C=O-groups into the structure of humics. The objectives of this study were to synthesize and characterize structure carbonylated humic materials and to estimate biological and detoxifying ability of the obtained HD with respect to copper. It was expected that introduction of additional CO-groups in the structure of humics would enhance their complexing and/or redox properties and, hence, would lead to an increased detoxifying ability of humic derivatives with respect to heavy metals.

2. MATERIALS AND METHODS

Humic acids (HA) of oxidized brown coal of two Kyrgyz deposits (Kara-Keche and Kyzyl-Kiya) were used as parent material for subsequent modifications. Carbonylation was performed by the treatment of HA by formic acid (Hatterman-Koch reaction). Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. Oxygen contents were calculated as a difference. The H/C and O/C atomic ratios were derived from the contents of the elements calculated on ash- and water-free basis. The data of chemical analysis and molecular-mass distribution are presented in Table 1.

Size-exclusion chromatography (SEC) analysis was performed according to a previous report (2). Redox capacity of humic materials was determined using ferricyanide as oxidizing agent as described in previous issue (3). Biological activity of the preparations was estimated by bioassay technique with seedlings. Seedlings of wheat *Triticum aestivum* L. were used as a target, and a root length was used as a response.

Table 1. Elemental and functional group composition of the carbonylated derivatives

Sample	Content of elements on ash-free basis, % (mass)						Content of functional groups, mmol/g			
	C	H	N	O	H/C	O/C	Total acidity	COOH	Ar-OH	Ar-CHO
Humate Kara - Keche	65.5	3.9	1.0	29.6	0.71	0.33	7,54	3,84	3,70	1,70
Humate Kyzyl-Kiya	64.3	4.0	1.1	30.4	0.73	0.35	7.63	4.21	3.42	1.61
Carbonylated Kara - Keche	63.0	4.2	0.8	32.0	0.40	0.91	7,69	4,27	3,42	2,19
Carbonylated Kyzyl-Kiya	65.2	2.5	0.9	31.2	0.46	0.34	7.62	4.18	3.44	2.17

3. RESULTS AND DISCUSSION

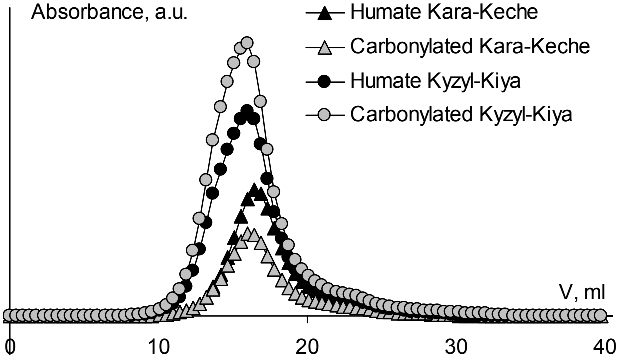

Figure 1. SEC-profiles of the parent humic material and carbonylated derivatives.

Chromatograms of all the parent humic materials and humic derivatives exhibited a sharp single symmetric peak. This finding confirms that the SEC-fractionation was conducted under conditions appropriate for the substances under study, as no artifacts such as ionic exclusion or specific adsorption were observed (2). That was also indicative for complete suppression of the surface negative charge of HA (ibid.).

Calculated values of molecular weight for all humic preparations are given in Table 2.

The HD were characterized with higher molecular weight Mw comparing with the parent humic material. This finding was evident for the partial polymerization occurred.

The values for redox capacity of the parent humic materials were 0.20, for Carbonylated Kara-Keche and Carbonylated Kyzyl-Kiya humics are 0.28 and 0.32 mmol/g respectively. All the HD possessed redox capacity considerably exceeding that of the parent humic materials. This finding was evident for increase in reducing capacity of humic derivatives comparing initial samples.

Table 2. Peak molecular weight Mp, number-average molecular weight Mn, weight-average molecular weight Mw, and polydispersity Mw/Mn of humic parent materials and obtained derivatives

Derivative Cipher	Mp, kD	Mn, kD	Mw, kD	Mw/Mn
Humate Kara-Keche	6.9	1.5	8.2	5.5
Humate Kyzyl-Kiya	8.5	1.9	12.9	7.0
Carbonylated Kara-Keche	8.1	0.8	9.5	11.9
Carbonylated Kyzyl-Kiya	9.2	0.9	13.7	14.3

The results on detoxifying ability activity of the humic derivatives are given in Figure 2.

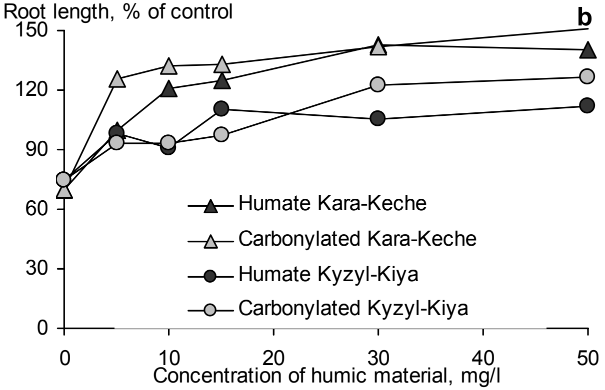

Figure 2. Dose-response relationships in the presence of 1 mg/L of copper(II) for the parent humic materials and carbonylated derivatives.

All the humic preparations studied significantly decreased toxicity of copper (Fig. 2). At their concentrations 30-50 mg/L no copper toxicity was registered. At low concentrations of HA (5-15 mg/L) the tendency was equal or slightly higher than that of the parent sample was observed.

The calculated values of the toxicological binding constants of the humic materials studied with copper(II) are given in Table 3.

It should be noted, however, that in all the cases of the carbonylated derivatives examined strongly pronounced toxicity was observed (Figure 3) what made incorrect calculations of toxicological binding constant K_{OC}^{tox}.

Table 3. The toxicological constants of copper binding to CHP-K$^+$ and its derivatives

Derivative Cipher	K_{OC}^{tox}, L/kg
Humate Kara-Keche	3.0×10^5
Humate Kyzyl-Kiya	8.7×10^5
Carbonylated Kara-Keche	4.0×10^5
Carbonylated Kyzyl-Kiya	5.0×10^5

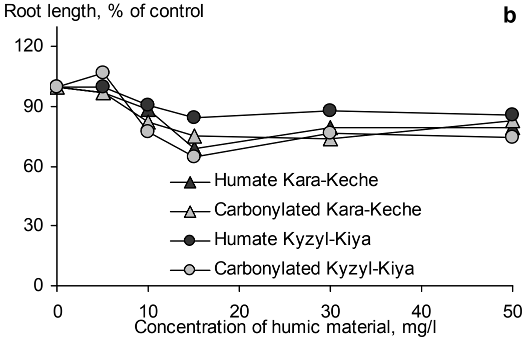

Figure 3. Dose-response relationships for the parent humic materials and HD.

4. CONCLUSIONS

The unknown phenomenon of humics biological activity was observed when carbonylated derivatives served as copper antagonist. Both copper and humic derivatives showed marked toxicity towards plant seedling when applied solely, while combined application of the above substances resulted in pronounced beneficial effect on plant growth.

ACKNOWLEDGEMENTS

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