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Auxin-Like Activity of Different Fractions of Coal Humic Acids

Introduction

In series of papers published since 1914 (Bottomley, 1914; Piccolo et al., 1992) it has been shown that HS enhance plant growth by exhibiting auxin-like activity. It was demonstrated that HS do not contain auxin structures. Hence, the mechanism of auxin-like activity of HS is still unclear. The objective of this study was to estimate auxin-like activity of narrow fractions of coal humic acids (HA) and establish its relationship to the structural features of HA.

Material and Methods

Coal humic acids (HA) were isolated from Gusinoozersk deposit of brown coal using 0.1 M NaOH extraction procedure according to (Lowe, 1992). Fractionation of HA (CHA-GL02) has been performed as described elsewhere (Stevenson, 1985) and included separation into hymatomelanic HA (CHM-GL02), a sum of brown and grey HA (CHR-GL02), and fractions of brown (CHB-GL02) and grey (CHG-GL02) HA. The obtained fractions of HA have been characterized using size-exclusion chromatography and ^{13}C NMR spectroscopy as described in (Perminova, 1999) and (Hertkorn et al., 2002), respectively. Carbon distribution and peak molecular weight of the HA fractions are presented in Table 1.

Auxin-like activity of different fractions of HA has been estimated using bioassay with coleoptiles of wheat (*Triticum aestivum*) seedlings (Handbook..., 1994). Briefly, wheat seeds were germinated in the dark at 25°C for 72 hours. Then coleoptiles of similar length (about 5 mm) were cut and spited on glass capillaries (3 coleoptiles on a capillary). Upper part of coleoptiles of 4 mm length was preliminary removed to avoid influence of endogenous auxin. Then the length of coleoptiles was measured and capillaries with beaded coleoptiles were placed into the Petri plates added with 5mM K-phosphate buffer at pH 6.0 (blank) or with a solution of HS preparations in phosphate buffer. Then coleoptiles were grown at 25°C for 72 hours and the length of coleoptiles was measured again. The relative increase of coleoptiles length was used as a response. The concentration of HS varied in the range of 5 to 100 mg/L.

Results and Discussion

All the HA samples tested caused the beneficial effect on coleoptiles growth at all the concentrations tested (Fig. 1). This fact confirms the auxin-like activity of HA.

Maximum stimulating activity was observed at the concentration of 10 mg/L HA. The registered effects for all the fractions of coal HA at the specified concentration are presented in Table 1.

As can be seen from Table 1, the efficicency of different HA fractions was similar except for

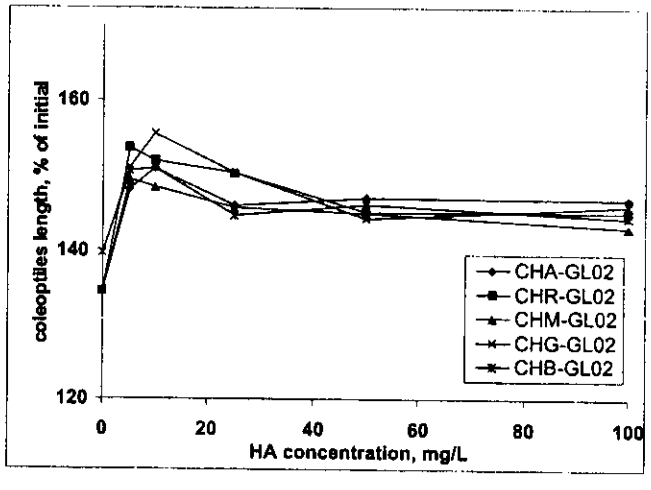

Fig. 1. Influence of different fractions of coal HA on the wheat coleoptiles growth

Table 1. Carbon distribution, peak molecular weight (MW), and auxin-like activity of the fractions of coal HA

HA sample	Carbon distribution, % of total C							MW, KDa	Effect[1], %
	C=O	COO	$C_{Ar}O$	C_{Ar}	$C_{Alk}O$	CH_3O	C_{Alk}		
CHA-GL02	5.1	13.9	8.2	51.3	3.4	1.3	14.3	22.3	151
CHR-GL02	4.8	12.3	8.5	43.9	6.5	1.6	20.3	27.4	152
CHM-GL02	4.4	17.1	7.8	43.7	7.8	2.2	14.4	19.5	149
CHG-GL02	4.4	10.5	10.8	38.5	6.9	1.2	24.1	34.0	156
CHB-GL02	6.7	15.1	7.5	51.5	2.7	0.9	13.0	20.8	151

[1] – *Effect has been estimated as the coleoptiles length, % of initial. For the blank that value was 35%.*

hymatomelanic and grey HA. The highest effect was observed for the grey HA characterized with the lowest contents of such fragments as carboxylic (COO^-) and aromatic (C_{Ar}) groups and with the highest MW. On the other hand, hymatomelanic HA possessing the highest contents of alkoxy ($C_{Alk}O$) and methoxy (CH_3O) groups and the minimum value of MW, exhibited the lowest beneficial effect on the coleoptiles growth. Statistical analysis of the data set on HA properties and their auxin-like activity have revealed a significant (P =95%) negative relationship between the contents of carboxylic groups in the HA preparations and the value of the beneficial effect ($r = -0.96$). Taking into consideration a determining role of carboxylic groups in formation of the surface negative charge of HA and the natural negative charge of cell surface, it could be suggested that this is adsorption of HA onto the coleoptiles surface that determines the auxin-like activity of HA.

References

A handbook of plant physiology. 1985. Mokronosov (Ed.). Moscow, Moscow State University Publisher, 184p. (in Russian).

Bottomley, W.B. 1914. Some accessory factors in plant growth and nutrition. *Proc. of the Royal Society of London (Biology)* **88**, 237-247.

Hertkorn, N., A.B. Permin, I.V. Perminova, D.V. Kovalevskii, M.V. Yudov, A. Kettrup. 2002. Comparative analysis of partial structures of a peat humic and fulvic acid using one and two dimensional nuclear magnetic resonance spectroscopy. *J. Environ. Qual.*, **31**, 375-387.

Perminova, I.V. 1999. Size-exclusion chromatography of humic substances: complexities of data interpretation attributable to non-size exclusion effects. *Soil Sci.*, **164** (11), 834-840.

Piccolo, A., S.Nardi, G. Concheri. 1992. Structural characteristics of humus and biological activities. *Soil Biology & Biochemistry*, **24**, 273-380.

Stevenson, F.J. 1985. Geochemistry of Soil Humic Substances. - In: Humic substances in soil, sediment and water. Aiken G.R., McKnight D.M., Wershaw R.L., MacCarthy P. (Eds.), N.Y., John Wiley & Sons, p. 13-52.

Lowe, L.E. 1992. Studies on the nature of sulfur in peat humic acids from Froser River delta, British Columbia. *Sci. Total Environ.*, **113**, 133-145.

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