



Influence of metal ions binding on free radical concentration in humic acids. A quantitative electron paramagnetic resonance study

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Abstract

The influence of metal ions, e.g. Co(II), Cu(II), Mn(II), Ni(II), Fe(II), on free radical concentration in humic acids isolated from soil, peat and compost was investigated by electron paramagnetic resonance (EPR). The results show that metal ions with unfilled d-shell exhibit antiferromagnetic interactions with semiquinone radicals. Moreover, coordinated metals shift the quinone–semiquinone–hydroquinone equilibrium in the macromolecular matrix of humic acids. A strong decrease of semiquinone radical concentration in humic acid–metal complexes is observed. This effect is caused by interactions of metal ions with oxygen-containing stable radicals occurring in the aromatic systems of humic acids. Furthermore, the effect of metal coordination on free radical concentration in humic acids–metal complexes depends on the humic acid origin. FTIR spectroscopy was also used as an additional tool for studies of the metal ions interactions with carboxylic groups. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Humic acids (HA) are widespread constituents of natural environments. They are capable of metal binding, buffering and sorption of various organic and inorganic substances. Structural transformations during ageing of humic acids lead to the decrease in the number of aliphatic and carboxylic groups, and to the increase in the number of polyphenolic and quinone units. The equilibria formed between structural polyphenolic and quinone units result in semiquinone intermediates bearing unpaired electrons which can be investigated using electron paramagnetic resonance spectroscopy (EPR) (Senesi and Loffredo, 1999). The quinone–semiquinone–hydroquinone equilibria are sensitive to various chemical factors such as radiation, pH and redox reactions

(Senesi, 1990, 1992). Further, the carboxylic, phenolic and quinone groups of HA are involved in metal complexation. Therefore, interactions of humic acids with metal ions can also play a significant role in shifting the reactions equilibria (Jerzykiewicz et al., 1999a,b; Jezierski et al., 1998, 2000a, b). The effect of metal binding is observed as a change in the free radicals structure and/or spin concentration. Studies of metal ions–free radical interactions could lead to valuable indices of metal pollution of natural organic matter. In this work we study the influence of metal ions on the free radical concentration for selected HA of various origins.

2. Experimental

Humic acids were extracted from different sources: (1) compost HA from compost produced by the Municipal Composting Plant in Zabrze (Upper Silesia, Poland); (2) soil HA- from brown soil (Lower Silesia, Poland); (3) peat HA from lowmoor peat (Odra river lowland near Wrocław, Poland). The isolation were carried out using

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standard IHSS (International Humic Substances Society) procedure (Swift, 1996; Stevenson, 1982; Hayes, 1985) by shaking the compost samples in a solution of NaOH and $\text{Na}_4\text{P}_2\text{O}_7$ and centrifugation, acidification to pH 1.0 with HCl. The HA–metal complexes (HA–M), were obtained for Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) ions. Acetate salt solutions of the metals were applied. Samples of humic acids (0.5 g) were treated with 0.2–5 cm^3 of 0.05 M metal acetate solutions to obtain complexes of different metal concentration. The suspension was stirred at 60 °C for 15 min then left for 24 h to saturate with each metal ion. The resulting complexes were filtered off and washed with 50 cm^3 of distilled water and dried at 80 °C for 4 h. The dry powders containing HA–metal complexes were analysed by EPR.

The effective amount of metal uptake was assessed in each case by AES ICP measurements (Atomic Emission Spectroscopy Inductively Coupled Plasma) method (ARL 3410 instrument). Electron paramagnetic resonance (EPR) spectra were obtained with Radiopan SE and Bruker ESP300E spectrometers operating at X-band frequencies at room temperature. The solid samples were placed in quartz tubes of 5-mm diameter for EPR measurements. The Bruker spectrometer with a 100 kHz magnetic field modulation was equipped with a Bruker NMR ER 035M gaussmeter and a Hewlett-Packard HP 5350B microwave frequency counter. A Li/LiF standard was used for g-value calibration; 4-hydroxy-TEMPO and Reckitt's ultramarine were used as standards of spin concentration. The quantitative EPR technique was applied: microwave power 2 mW, modulation amplitude 1 G, 20.0 mg sample, standard quartz tubes. The standard peat and Leonardite HA extracted and distributed by IHSS were used as additional standards of free radical concentration. FT infrared spectra (FT–IR) were recorded on Bruker 113v FT–IR spectrometer on KBr pellets using 1 mg of HA–M per 400 mg KBr. Elemental analysis (C, H, N) was performed on a Perkin-Elmer 2000 instrument. Carboxyl groups content were established by titration (Swift, 1996).

3. Results and discussion

3.1. Influence of metal ions on semiquinone free radicals

We studied the effect of metal ion complexation on the concentration of free radicals in humic acids (Fig. 1). The results show that the concentration of free radicals decreases sharply with metal ion concentration. Moreover, the effectiveness of the phenomenon strongly depends on the metal ion. On the basis of data presented in this paper and of earlier work (Jeziński et al. 2000a, b) it is concluded that complexation of HA with d^n metals ions for which $n < 10$ (Mn(II), Fe(II), Co(II), Ni(II) and Cu(II)) induces a decrease of spin concentration in the

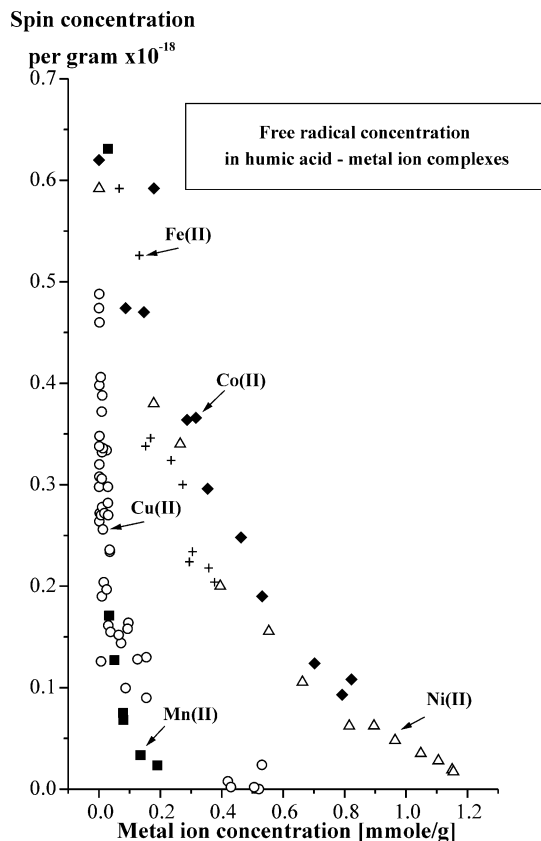


Fig. 1. Correlation between semiquinone free radical concentration and metal ions [Mn(II), Ni(II), Co(II), Fe(II), Cu(II)] content for peat humic acid–metal complexes.

humic acid–metal ion complex. In contrast to the above observation, in case the of Zn(II), Cd(II), Ca(II) and other diamagnetic ions of d^{10} or d^0 configuration, the increasing effect of metal coordination on the semiquinone free radical concentration is observed (Jeziński et al., 2000a,b).

A strong spin quenching effect is characteristic for Cu(II) and Mn(II) complexation as shown in Fig. 1. In contrast to the suggested reduction of copper(II) to copper(I) in polyphenolic-quinone systems (Halliwell and Gutteridge, 1999), this effect is interpreted as an anti-ferromagnetic interaction (Bencini and Gatteschi, 1990) of the copper(II) d-shell with semiquinone unpaired electrons. Therefore, this co-ordination is not a redox process. It was found that simple 1 M HCl washing out of metal ions from HA–M(II) complexes results in complete recovery of spin concentration originally present in uncomplexed HA. Moreover, a supporting argument comes from the strong radical quenching by Mn(II) and other metal ions [Ni(II), Co(II) and Fe(II)] that have no redox properties towards the humic acid systems. The very low Mn(II) concentration (0.2 mmol/g HA) decreases the spin concentration to the non-measurable

minimum, whereas for Ni(II)–HA complexes the same effect is observed for much higher metal concentration (1.2 mmol/g HA). In the case of Ni(II) complexation, the tendency of decreasing number of spins/g is easily observed for HA from various sources.

3.2. Influence of metal ions on humic acids from different sources

We studied the effect of the Ni(II) complexation on the concentration of free radicals in humic acids from peat, compost and soil (Fig. 2). The results show a decrease of radical concentration with Ni(II) levels. Moreover, this decrease is stronger for compost HA than for soil and peat HA. This source effect could be explained by different ability of containing semiquinone free radicals humic acids to open up to interact with metal ions. Indeed, semiquinone free radicals in material of low carboxyl group content; see Table 1; (2.76 meq/g) and low C/N ratio (10.90), such as compost HA, are highly sensitive to metal interaction. The semiquinone free radical quenching effect for that HA is stronger than observed for soil and peat HA with higher COOH

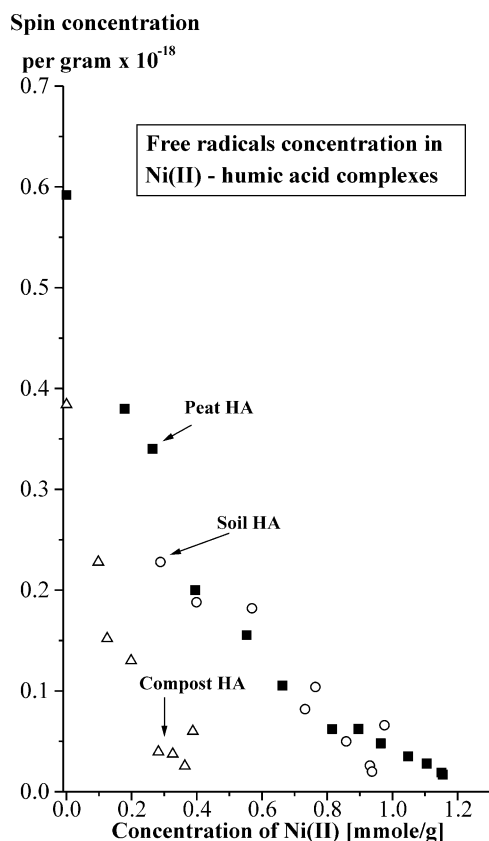


Fig. 2. Correlation between semiquinone free radical concentration and Ni(II) content in Ni(II) complexes with humic acids from peat, soil and compost.

Table 1

Acidic functional group concentrations and C/N ratio for HA extracted from different sources: peat, soil and compost

	C/N	Total acidity	–COOH	–OH
		(meq/g)		
HA peat	21.16	8.14	3.11	5.03
HA soil	17.10	4.87	2.67	2.20
HA compost	10.90	5.66	2.76	2.90

content (3.11 meq/g, peat) and higher C/N ratio (17.10, soil, 21.16 peat). A lower carboxyl group content in HAs induces better access of other groups, for example phenolic groups, to metal ions and shifts quinone–semiquinone–hydroquinone equilibria towards hydroquinone groups that results in a decrease of radicals concentration.

3.3. Infrared spectroscopy of humic acid–metal complexes

Infrared spectroscopy has been used for the assessment of HA functional group interactions with metal ions. The FT–IR spectra of uncomplexed HA (Fig. 3) show typical absorbances for this type of material. The changes observed between HA and HA–M spectra concern especially stretching frequencies of carboxyl groups (1710–1635 cm⁻¹) (Inbar et al., 1990). These absorbances in humic acids–metal ion complexes change to one unresolved band shifted to lower wavenumber (1600 cm⁻¹). Simultaneously, the band of $\nu(\text{C–O})$ at 1230 cm⁻¹ for HA shifts to the values around 1380 cm⁻¹ for HA–M complexes. The value of Δ parameter (defined as $\nu(\text{C=O}) - \nu(\text{C–O})$) for complexes in the range 216–240 cm⁻¹ indicates formation of unidentate complexes (Nakamoto, 1989).

3.4. Carboxyl groups in humic acids–metal complexes

We observed that for the samples of peat and compost HA with the highest Ni(II) concentration and with maximally decreased free radicals concentration still 2.2 (peat HA) and 1.8 (compost HA) meq/g COOH groups are unbound. These groups are probably hidden and inaccessible for metal ions coordination in the macromolecular matrix of HA. On the other hand, for the sample with the highest Mn(II) content (0.7 mmol/g) only 0.42 meq/g COOH groups are involved in the investigated interaction, so excess of Mn(II) is bound with groups other than carboxyl, probably phenol groups. It could explain the high sensitivity of Mn(II) coordination on semiquinone free radicals concentration–shifting of the equilibrium quinone–semiquinone–hydroquinone.

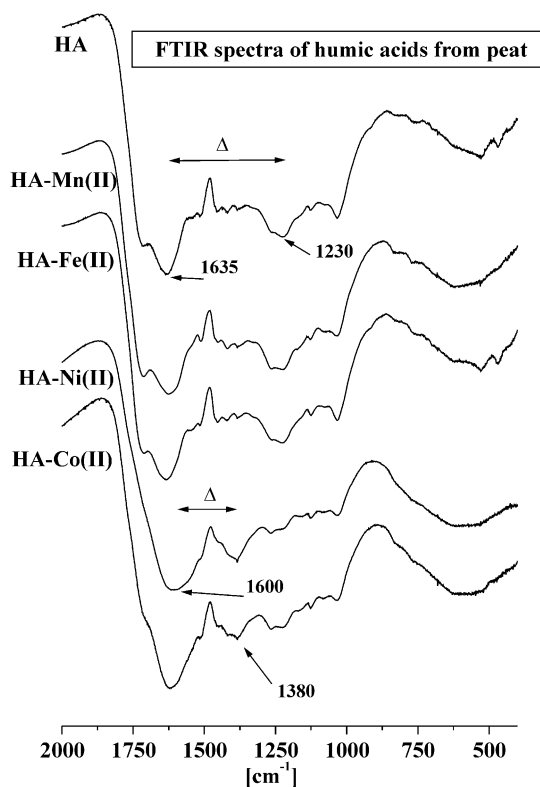


Fig. 3. Fourier transformed infrared (FT-IR) spectra of peat-humic acids and complexes with Mn(II), Fe(II), Ni(II) and Co(II).

4. Conclusions

Quantitative EPR spectroscopy is useful to gain information on the concentration of free radicals in humic acids and humic acid complexes. The strongest effect of semiquinone radical quenching by metal ions in HA–M complexes is observed for Mn(II) and Cu(II) ions while for Ni(II), Co(II) and Fe(II) the effect is smaller but significant. The decreasing tendency of semiquinone radical concentration is due to humic acids interacting with d^n ($n < 10$) metal ions and depends on the HA origin. We presume that the observed differences are associated with the different ability of humic acids to open up to interact with metal ions, with the centres being semiquinone free radical.

Acknowledgements

This work was financially supported by Komitet Badan Naukowych, project 6PO4G 044 18.

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