

# Electron paramagnetic resonance (EPR) studies on stable and transient radicals in humic acids from compost, soil, peat and brown coal

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## Abstract

Quantitative EPR method was applied to characterise four types of humic acids (HA) derived from composts, soil, peat and soft brown coal. For each sample of HA the level of native (indigenous) radicals was estimated. Interactions of the HA with various gaseous agents and organic solvents were investigated. Strong effects of gaseous ammonia and aliphatic amines on spin concentration enhancement were observed; additionally, higher values of *g*-value were found to be associated with the formed ‘transient’ radicals. Correlation of copper(II) ions uptake by different HA with effect diminishing primary spin concentration was established. It was recognised that the radical centres, which are enhanced by ‘ammonia effect’ are quenched in the formed HA-Cu(II) complexes. The both opposite effects are competitive from each other, where ‘copper(II) quenching effect’ prevails. Reaction of nitrogen dioxide with the humic acids was also examined. The presence of diketones and/or other compounds with active methylene group results in formation of the iminoxy radicals; these radicals are immobilized in the solid (macromolecular) matrix of the humic acids. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Humic acids; EPR; Free radical; Humic acids-copper(II) complex; Semiquinone radical; Iminoxy radical

## 1. Introduction

In the natural environment are taking place numerous free-radical reactions, which play an

important role in polymerisation and depolymerisation processes of organic matter transformations. The humic substances stabilise organic (mainly semiquinone) radicals in their macromolecular matrix. Therefore, the *g*-value and concentration of the stable radicals may be used as a source of information concerning physicochemical

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processes in the environment [1–3]. The free radical concentration depends on numerous natural (environmental) and artificial (laboratory) factors. Humification of organic matter, particularly in solid waste composts and in soil, as well as some features of chemical humic matter constitution can be followed by EPR method [4–9]. The knowledge of physicochemical properties of humic substances and their structural changes during natural maturation of the source organic matter gives basis for the assessment of their quality and utility [8,9]. Two classes of the radicals in humic substances are recognised [5]:

1. a group of the stable ('native' or 'indigenous') radicals;
2. a group of transient (short-lived) radicals.

Radicals in humic substances are sensitive to pH, ionic strength, redox properties and interactions with metal ions [5–9]. Termination of the various free radical reactions proceedings during decomposition of the organic matter leads to formation of the EPR-detected stable radicals; generated upon degradation processes polymeric substances like: polyphenols, melanins, melanoidins and other Maillard polymers, humic and fulvic acids, etc., act as effective spin traps. This gives the basis of the EPR approach.

The aim of the work was to investigate the humic acids derived from four different sources, i.e. compost, soil, peat and soft brown coal with the use of EPR spectroscopy. Different chemical agents (gaseous oxygen, nitrogen, ammonia and nitrogen dioxide, *n*-propylamine, and other organic solvents, copper(II) ions) were used to characterise chemical properties of the paramagnetic radical centres.

## 2. Experimental

The experiments were carried out on humic acids (HA) isolated from solid wastes composted under different conditions (samples assigned as HA-C) [9], soils (HA-S; originated from Cambic Podzol, Gleyic Podzol and Chernozem) and peat (HA-P, lowmoor peat) and from Tertiary earthy soft brown coal (HA-EBC).

Isolation of HA was achieved using standard IHSS procedures [10] by extraction with NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solutions and subsequent HCl precipitation, and in case of HA-EBC the extraction of humic acids was carried out with 0.1M NaOH. The prepared HA samples, after liofilisation, constituted materials for EPR and other physicochemical studies.

The samples of HA-P and HA-EBC were used to obtain the HA–Cu(II) complexes. The finely powdered HA samples (500 mg) were suspended in 50 cm<sup>3</sup> of different concentration Cu(II) acetate or sulphate solutions and shaken for 24 h at room temperature. Effective amount of Cu(II) uptake was assessed in each case by ICP method.

Electron paramagnetic resonance (EPR) spectra were obtained with Radiopan SE and Bruker ESP300E spectrometers operating at X-band frequencies at room temperature. For measurements the solid samples were placed in quartz tubes of 5-mm diameter. The Bruker spectrometer with a 100 kHz magnetic field modulation was equipped with Bruker NMR gaussmeter ER 035M and Hewlett-Packard microwave frequency counter HP 5350B. 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 (QEPR) was applied (microwave power 2 mW, modulation amplitude 1 G, 20.0 mg sample, standard quartz tubes, etc.).

EPR measurements were carried out:

1. for the HA and HA-Cu(II) complexes under atmosphere of gaseous ammonia, oxygen or nitrogen, respectively, flowing over the samples in the quartz tubes placed in the resonance cavity,
2. with liquid *n*-propylamine added (amount of amine added was equivalent to the sample mass); in the same way, prior to measurements, samples were treated with the other organic solvents (mono-, di- and tri-*n*-propylamine, tri-ethylamine, ethanolamine, ethylenediamine, pyridine, quinoline, *N*-methylimidazole, chloroform, mesitylene, tetralin, aliphatic thiols and others)
3. for the HA samples treated with gaseous nitrogen dioxide (5% v/v in air) for 5 h.

Infrared spectra (IR) were recorded with an FTIR Bruker 113v spectrometer, on KBr pellets (1 mg sample per 400 mg KBr).

### 3. Results and discussion

In the HA from sources of lower humification degree (HA-C and HA-S) generally the observed values of  $g$ -value are little higher while spin concentrations are up to 10-fold lower compared with these parameters of HA originating from peat and brown coal (HA-P and HA-EBC, respectively, see Table 1). All investigated materials are characterised by single-line EPR spectra, which are associated with various oxygen centred radicals. They are mainly of semiquinone nature, but chemical environment in general and interaction of the radical centres with others oxygen containing groups (e.g. mainly OH, COOH, CO) causes shifting the observed average  $g$ -value to higher values [11]. Nitrogen atmosphere has no influence on the  $g$ -value value and spin concentration, while in oxygen atmosphere spin concentration increases insignificantly by about 3–10%. It indicates that the observed signals for the investigated HA are not originated from the radicals derived from polyaromatic units [12]. However, these radicals are very sensitive to the gaseous or liquid chemical agents strongly interacting with the HA.

#### 3.1. Interaction of HA with solvents and gaseous media.

Gaseous media and solvents influence free radical concentration and arrangement in these materials. Comparison of the free radicals concentration in HA treated with different solvents with those data for starting materials allow to recognise the general types of interaction between HA matrix and solvent molecule. The interaction of examined solvents possessing basic nitrogen causes enhancement of spin concentration of the HA in the following order: monoalkylamines containing small or linear aliphatic chains (ethylamine, *n*-propylamine, *n*-nonylamine) > monoalkylamines containing secondary or tertiary aliphatic groups (sec-butylamine, tert-butylamine)  $\cong$  pyridine > dipropylamine > aniline. However, the solvents without basic nitrogen in the structure as well as tertiary amines or possessing larger substituent give no effect on the spin concentration of the samples: mesitylene, tetralin, aliphatic thiols, quinoline, dimethylhydrazine, as well as tripropyl- and triethylamine, *N,N,N',N'*-tetramethylethylenediamine, cyclohexylamine. The above-presented data can be interpreted under supposition of two effects, which are responsible for the observed increase of spin concentration: basicity of the solvent and steric properties of the solvent molecule. The observed effect is a summary of the two above factors. Solvent basicity acts as increasing-value of free radical concentra-

Table 1

EPR data (spin concentration and  $g$ -value for free radicals) of HA derived from compost, soil, peat and soft brown coal, before treatment and after treatment with gaseous ammonia

Humic acids sample designation	Raw samples (air atmosphere)		Ammonia atmosphere		
	$g$ Value	Spin concentration $\times 10^{-18}$	$g$ Value	Spin concentration $\times 10^{-18}$	Multiplicity of spin concentration enhancement
HA-C	2.0031–2.0037*	0.008–0.02*	2.0040–2.0044*	0.03–0.16*	4–8
HA-S	2.0035–2.0041**	0.04–0.14**	2.0038–2.0041**	0.1–0.3**	3–6
HA-P	2.0033	0.151	2.0042	0.508	3
HA-EBC	2.0035	0.35	2.0045	1.60	5

\* Depending on compost maturity [9].

\*\* Depending on the soil; the lowest spin concentrations were observed for Podzol HA, the highest for Chernozem HA.

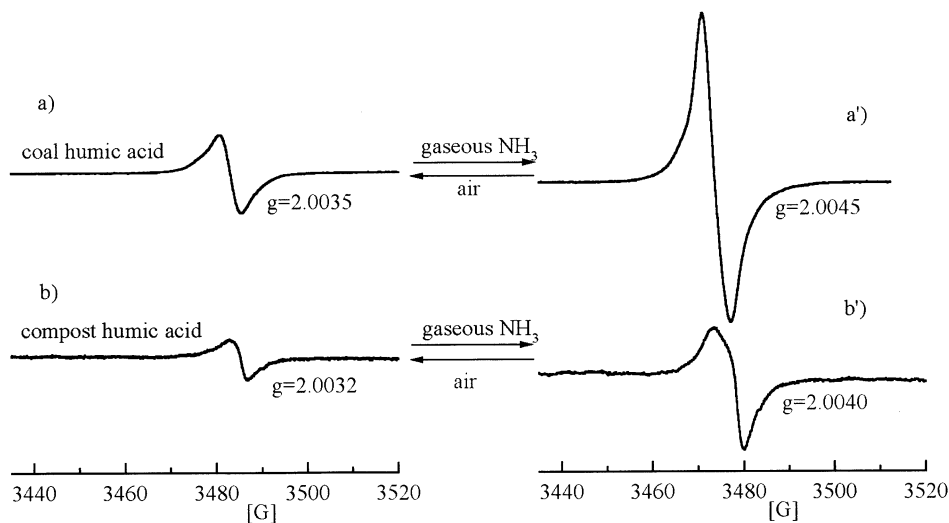


Fig. 1. Quantitative EPR spectra of HA extracted from compost (a, a') and soft brown coal (b, b'), at 20°C, before (a, b) and after ammonia treatment (a', b').

tion. However, the more bulky structural constitution of the solvent molecule limits accessibility to the HA matrix.

Sterically small gaseous ammonia molecule, possessing ability to easy HA matrix penetration gives similar spin concentration enhancing effect to the effect of aliphatic amines. This 'ammonia effect' (Fig. 1) is attributed to quinone-hydroquinone-semiquinone equilibria (involving anionic forms of the radicals) [5,6] and opening-up of HA structure because of interaction with the base. The interaction of ammonia with HA was found to be fully reversible. Namely, the multiple interchange of ammonia to air atmosphere and reverse give repeatable results for the treated and untreated sample, respectively. The enhancement of the radical concentration caused by 'ammonia effect' is from four to eight folds that compared with the value of raw samples. This effect is even more pronounced for HA treated with stronger base easily penetrating the HA structure like *n*-propylamine. It was also noticed that spin concentration enhancing effect is accompanied by *g*-value increase (Table 1). Increase of *g*-value is HA nature dependent and it is the highest for HA-EBC.

On the other hand, the HA interaction with copper(II) ions results in formation of complexes exhibiting lower values of spin concentration in comparison to starting materials. Similar phenomenon was observed recently for the soils contaminated by copper(II) [13]. Systematic investigations on the HA-Cu(II) complexes of different copper content revealed that higher copper(II) ions uptake inversely correlates with spin concentration (Fig. 2). The pathway of this trend, as illustrated in Fig. 2, is HA source dependent. Namely, copper(II) interaction in HA-P-Cu(II) complexes has more pronounced effect on the spin quenching compared with HA-EBC-Cu(II) counterparts. It is reflected by the much lower copper(II) content in HA-P-Cu(II) complex required for achieving similar level of spin concentration decrease than in HA-EBC-Cu(II) complex. The course of the respective line for HA-EBC-Cu(II) complex proceeds above the line of HA-P-Cu(II) complex over the investigated copper(II) concentration range (0–5%).

The EPR parameters of the formed HA-P-Cu(II) complexes are:  $g_z = 2.270$  and  $A_z = 137$  G. According to the EPR parameters and changes in FTIR spectra (relative intensities of absorbances at about  $1720\text{ cm}^{-1}$  and  $1630\text{ cm}^{-1}$ , as well as at

1260  $\text{cm}^{-1}$  and 1230  $\text{cm}^{-1}$ ; see Fig. 3 for investigated HA-P-Cu(II) complexes) confirm Cu(II) coordination by phenolic and carboxylic groups, which agrees with the earlier evaluations [6,14]. The novel aspect of the HA-Cu(II) coordination observed in this work is the recognition of direct relation of diminishing free radical concentration

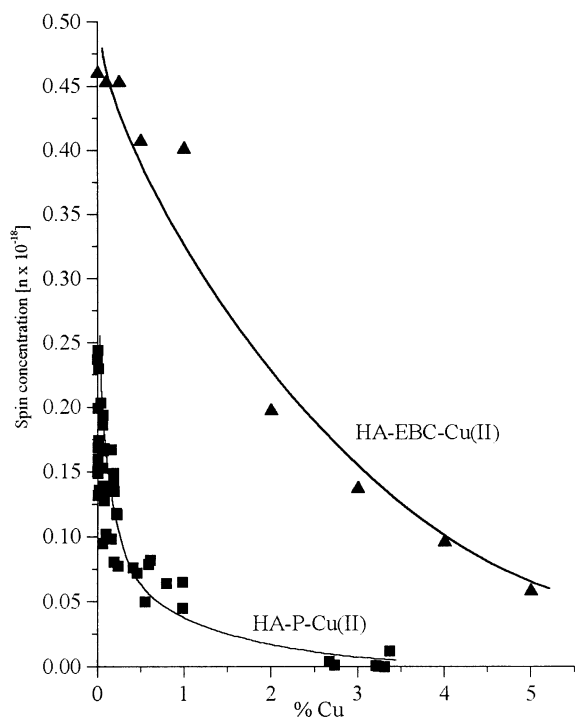


Fig. 2. Correlation of free radical spin concentration of peat (HA-P) and soft brown coal (HA-EBC) copper(II) complexes versus Cu(II) concentration.

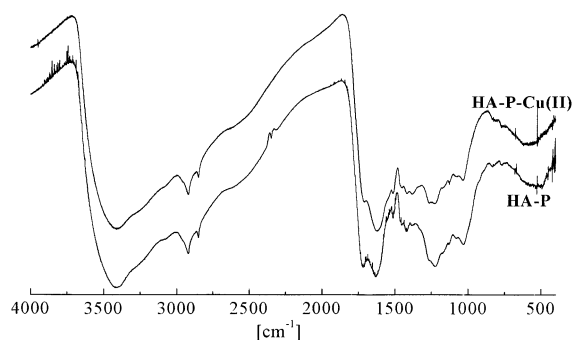


Fig. 3. FTIR spectra of peat HA-P and HA-P-Cu(II) complex.

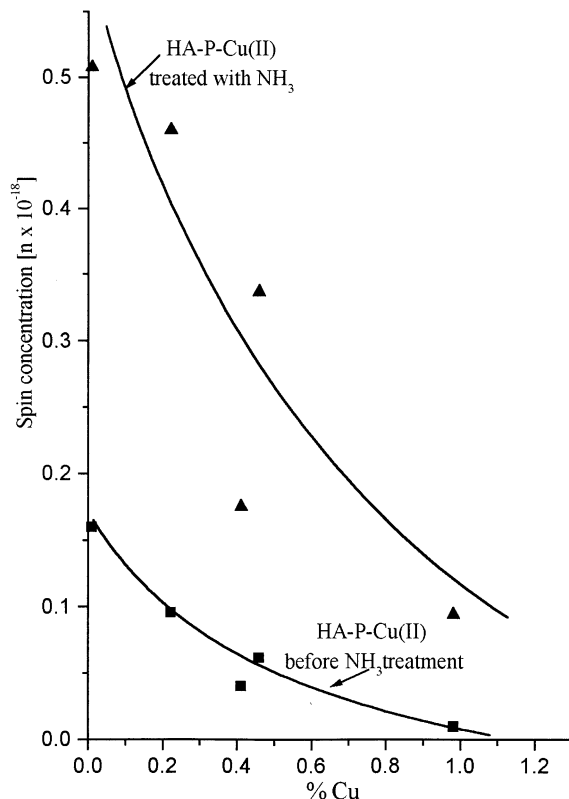


Fig. 4. Correlation between free radical spin concentration and Cu(II) content in HA-P-Cu(II) (lower curve) and HA-P-Cu(II) treated with ammonia (upper curve).

in the HA parallel to increasing amount of Cu(II) uptake. Moreover, this effect was found to be reversible; removal of copper(II) from the HA-Cu(II) complex using 1 M HCl solution results in complete recovering of the primary radical concentration in HA. Authors suggest similar chemical nature of oxygen-containing active sites responsible of copper(II) binding and of interacting with ammonia molecules. Formed HA-Cu(II) complexes cause spin concentration quenching while HA- $\text{NH}_3$  interaction products give rise of spin abundance. Because of the opposite directions of these effects as well as of HA-Cu(II) complexes higher stability, the 'ammonia effect' on HA-Cu(II) complexes gets gradually smaller with an increase of copper(II) bound to HA (Fig. 4). Summary result of the two competitive effects, of which 'copper(II) quenching effect' prevails, is

particularly well demonstrated for the samples containing higher percentage of copper(II). In case of HA-P-Cu(II) in the samples of Cu(II) content above 0.7% the ‘ammonia effect’ and ‘copper(II) quenching effect’ gives summary smaller spin concentration than it is present for the raw untreated HA-P (upper line in Fig. 4). Additionally, Cu(II) binding to HA within active oxygen containing centres, is not only associated with free radicals quenching but it also causes insignificant decrease of  $g$ -value (by 0.0002).

It is worthwhile to note that the higher  $g$ -value in HA-S and HA-C is rather associated with radicals rich in oxygen groups. It is characteristic that maturation of composts under good aeration conditions leads to increase of  $g$  found for HA-C (2.0031–2.0037). The same phenomenon occurs in nature, as it was observed for Podzol HA obtained from the soil of higher aeration (Cambic Podzol) for which  $g$  equals 2.0041, while for HA obtained from higher moisture soil (Gleyic Podzol, where aeration process is partially restricted)  $g$  is lower and it equals 2.0035. We presume that the radicals associated with oxygen rich structural units more effectively interact with ammonia (when their concentration increases, effective  $g$  also increases) and Cu(II) (their concentration decreases, effective  $g$  decreases).

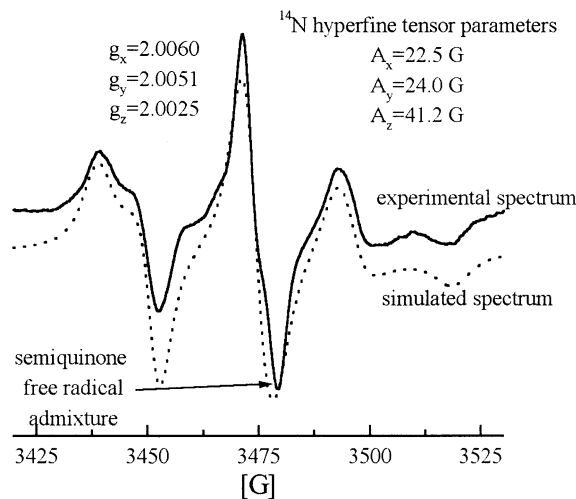


Fig. 5. EPR spectra at 20°C of iminoxy radical formed during reaction of HA-P with  $\text{NO}_2$ . Experimental spectrum, solid line, simulated, dashed line.

### 3.2. Interaction of HA with $\text{NO}_2$

Reaction of  $\text{NO}_2$  with the investigated materials gives the new type of radicals. It is well known that reaction of  $\text{NO}_2$  with compounds having a methylene or vinyl group adjacent to the carbonyl group leads to formation of iminoxy  $\sigma$ -radicals ( $\text{R}_1\text{R}_2\text{C}=\text{NO}$  type) [15]. The spectra obtained for solid HA treated with  $\text{NO}_2$  are characteristic for immobilized iminoxyls in solid matrices. The solid matrix is necessary for the observation of anisotropic  $^{14}\text{N}$  hyperfine splitting parameters (Fig. 5). The obtained spectra are interpreted on the basis of EPR spectra simulation procedure [16,17]. The iminoxyl signals were observed for all HA, however the maximum intensity of the resonances was found for HA from brown coal. High sensitivity of the reaction of HA with nitrogen dioxide leading to the iminoxy radicals allows to use the EPR spectroscopy as a analytical tool for characterisation active methylene groups in these substances.

## 4. Conclusions

Examinations of the EPR spectra of humic acids derived from different sources revealed two opposite effects on the signal characteristic achieved by chemical treatments. Molecules possessing electron donor capacity like gaseous ammonia and aliphatic amines, compared to the level of native (indigenous) radicals estimated for raw HA, exhibit strong effect on spin concentration enhancement. Concentration of the formed upon ammonia and amines treatment ‘transient’ radicals achieve saturation under gaseous ammonia or at about equivalent mass of amine added to HA. Lowering of ammonia molecular fraction in gas stream flowing over HA sample or lowering of amine mass fraction added is manifested by lower enhancement of spin concentration. Additionally, the formed ‘transient’ radicals are associated with shift of  $g$ -value to the higher values which indicates shift in equilibria quinone-hydroquinone-semiquinone. Interactions of gaseous ammonia and amines with HA are very weak as removal of these agents by air flow or evaporation leads to

recovery of the primary EPR characteristic of the samples. Opposite effect is observed in HA-Cu(II) complexes. The higher copper(II) ions uptake by HA gives more pronounced diminishing effect on primary spin concentration. Furthermore, the radical centres, which are quenched in the formed HA-Cu(II) complexes, are presumed to be the same, which are enhanced by ‘ammonia effect’. The both opposite effects are competitive from which ‘copper(II) quenching effect’ prevails. Namely, higher abundance of these centres by Cu(II) ions is manifested by stronger decay of ‘ammonia effect’. Reaction of nitrogen dioxide with the HA was also examined. The presence of diketones and/or other structural units with active methylene group is reflected in formation of the EPR detectable iminoxy radicals ( $R_1R_2C = NO$  type) immobilized in the solid HA with characteristic anisotropic  $^{14}N$  hyperfine structure.

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