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Characterization of humic acids extracted from sewage sludge-amended oxisols by electron paramagnetic resonance

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Abstract

In tropical soils, the high turnover rate and mineralization of organic matter (OM) associated with intensive agricultural use, generally leads to faster soil degradation than that observed in temperate climatic zones. The application of sewage sludge to the soils is one proposed method of maintaining soil organic matter, and is also an alternative method of disposing of this waste product. As well as containing large quantities of OM, sludge is also a significant source of supplementary nitrogen, phosphorus and other essential nutrients for plant growth. However, it is necessary to understand the qualitative and quantitative changes that take place in the OM in soil treated with sewage sludge. The approach of the present study was intended to identify possible structural changes caused by sewage sludge applications on soil humic acids (HAs). The HAs extracted from a Typic Achrortox under sewage sludge applications were characterized by electron paramagnetic resonance (EPR) spectroscopy. The soil samples were collected from a field experiment designed to evaluate the effects of different doses of sewage sludge on corn growth and development in Brazil. The sewage sludge originated from urban waste treated at the sewage sludge treatment station in the city of Franca, state of Sao Paulo, Brazil. The following soil treatments were studied: control (non-cultivated soil under natural vegetation (NC)), control soil amended with NPK (conventional corn fertilization) and four treatments N1, N2, N4 and N8 with applications of 3.5, 7, 14 and 28 Mg ha^{-1} of sewage sludge (dry matter), respectively. HAs were extracted from the surface layer using the methodology of the International Humic Substance Society (IHSS). Fe^{3+} and VO^{2+} ions complexed with HAs, and also semiquinone-type free radical (SFR) at concentrations of approximately 2.0×10^{18} spins g^{-1} HA were identified in EPR spectra. The levels of SFR were lower for treatments where the applied sewage sludge doses were equivalent to four and eight times the normal doses of N mineral fertilization, reaching values of 1.7×10^{18} and 1.24×10^{18} spins g^{-1} HA, respectively. The observed decrease in SFR content as sewage sludge dose was increased, was probably associated with the incorporation of less aromatic components into HAs originating from the sewage sludge.
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Keywords: EPR; Soil humic acid; Sewage sludge; Humification degree

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1. Introduction

The intense activity of microorganisms in tropical soils is well known. This activity results in a high turnover rate of organic matter (OM) and intense mineralization causing significant losses of soil organic carbon. This combined with intensive agricultural use generally leads to faster soil degradation than that observed in temperate climatic zones (Shang and Tiessen, 1997).

Tiessen et al. (1992) reported 30% losses of organic carbon in an Oxisol from northeastern Brazil, 6 years after conventional tillage. According to Silva et al. (1994), OM losses were 76% of the initial content in a Red-Yellow Latosol with 15–30% clay content, 41% for a Red-Yellow Latosol with >30% clay content and 80% for a Sandy Quartz Soil, after 5 years under soybean cultivation.

One of the current agricultural priorities is to sustain and maintain fertility levels of soil without damaging the natural ecosystem. Various alternatives, including no-till management systems and sludge application, have been recommended to achieve this objective (Bayer et al., 2002, 2001; Melo and Marques, 2000).

There is growing interest in the disposal of sewage sludge on agricultural soils as an alternative to its disposal in landfills (Melo and Marques, 2000). Sludge is a significant source of supplementary nitrogen, phosphorus and other nutrients essential for plant growth. Sewage sludge is also a good source of OM, the application of which improves fertilization levels and some physico-chemical properties of soils in land restoration (Melo and Marques, 2000; Piccolo et al., 1992), and thus it disposal on agricultural soils has been strongly promoted. In addition to these agricultural benefits, the incorporation of organic amendments and crop residues into the soil may also, in some situations, reduce emissions of CO_2 due to carbon fixation, which has implications for the mitigation of the greenhouse effect (Bayer et al., 2000a,b).

Furthermore, the application of organic amendments to soils decreases the anthropogenic impact on the environment by avoiding direct discharge of pollutant wastes. However, care must be taken to avoid off-site pollution by heavy metals and pathogens (Shanableh and Ginipe, 1999).

There is still a need to understand qualitative and quantitative changes that take place in the soil OM caused by agricultural management systems, and this has largely been carried out using spectroscopic techniques. The use of spectroscopic techniques, such as electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), amongst others, provides useful insights into the chemical composition of humic substances (Preston, 1996; Kogël-Knabner, 1997; González-Pérez et al., 2004). These techniques also permit the determination of parameters describing the humification stage, such as the degree of aromaticity, as revealed by solid-state ^{13}C NMR (Stevenson, 1994), and the semiquinone-type free radical (SFR) concentration as determined by EPR (Schnitzer and Lévesque, 1979; Novotny and Martin-Neto, 2002; Senesi, 1990). Electron paramagnetic resonance spectroscopy has been widely applied in the characterization of composted organic materials (Senesi et al., 1992; Jerzykiewicz et al., 1999) and to monitor soil (Jezierski et al., 1998) and air pollution (Jezierski et al., 1999). The EPR spectroscopy can provide unique information on the nature and concentration of organic free radicals present in humic substances. In addition, paramagnetic metal ions complexed with humic substances can be identified, and the type and symmetry of coordination sites can be investigated (Senesi, 1990).

In this study, humic acids from a typic Achrortox under sewage sludge applications were characterized by EPR spectroscopy. This approach was intended to identify possible structural changes in soil humic acids (HAs) caused by sewage sludge applications.

2. Materials and methods

2.1. Humic acids

Humic acids (HA) were extracted from the surface layer (0–20 cm) of a Typic Achrortox containing approximately 42% clay, 8% silt, 50% sand and 3.2% OM. Soil samples were collected 2 years after installation of a field experiment designed to evaluate the effects of different doses of sewage sludge on corn growth and development. The field experiment is located at the Experimental Farm of the Brazilian Agriculture Research Interprise (EMBRAPA) at the Center for Environmental Research in Jaguariúna, (Sao Paulo, Brazil). The area is located at 22°41′S, 47°W and 570 m altitude, in a subtropical region with a mean annual temperature of 22 °C, humid summers and dry winters. The sewage sludge originated from urban waste treated at the sewage sludge treatment station in the city of Franca (Sao Paulo). The following soil treatments were selected: control (non-cultivated soil under natural vegetation (NC)), control soil amended with NPK (conventional corn fertilization) and four treatments N1, N2, N4 and N8 (with sewage sludge applications). Treatment N1 was equivalent to the dose of nitrogen applied as conventional mineral fertilization

Table 1
Elemental analysis of HA samples (ash-free basis) from different treatments with and without (control and NPK) sewage sludge applications

Sample	N (%)	C (%)	H (%)	S (%)	O (%)	C/N	O/C[a]	H/C[a]
Control	3.71 ± 0.49	49.15 ± 0.58	4.98 ± 0.13	0.68 ± 0.17	41.48 ± 0.84	13.23	0.63	1.21
NPK	4.90 ± 0.44	50.50 ± 0.78	4.96 ± 0.13	0.37 ± 0.22	39.27 ± 0.85	10.30	0.58	1.18
1N	4.89 ± 0.44	49.91 ± 0.61	5.02 ± 0.11	0.40 ± 0.21	39.79 ± 0.70	10.21	0.60	1.21
2N	4.13 ± 0.38	49.75 ± 0.58	5.23 ± 0.10	0.77 ± 0.18	40.12 ± 0.65	12.05	0.60	1.26
4N	4.14 ± 0.37	48.64 ± 0.69	5.20 ± 0.10	0.76 ± 0.18	41.27 ± 0.77	11.76	0.64	1.28
8N	5.24 ± 0.55	49.28 ± 0.57	5.60 ± 0.24	0.51 ± 0.18	39.37 ± 0.82	9.41	0.60	1.36

[a] Atomic ratio.

in corn and the other doses were two, four and eight times the quantity of sewage sludge in treatment N1, corresponding to 3.5, 7, 14 and 28 Mg ha^{-1} of sewage sludge (dry matter), respectively.

The International Humic Substance Society (IHSS) method was used to extract humic substances from the soil (Stevenson, 1994). Briefly, the method includes extraction with 0.1 mol L^{-1} NaOH, using a soil: solvent ratio of 1:10. After centrifugation, the HAs were separated from the supernatant by precipitation with 6 mol L^{-1} HCl added to the extract, until a pH of 2.0 was reached. The precipitated HAs were separated by centrifugation, and purified by dialysis using Spectrapor membrane (size exclusion limit, 6000–8000 D) and finally were freeze-dried.

The elemental composition (C, H, N, S) of HAs was measured using a Fisons Instruments Elemental Analyzer EA 1110 and the data obtained are shown in Table 1.

2.2. Electron paramagnetic resonance (EPR)

All EPR measurements were performed using a Bruker EMX spectrometer operating at X-band (9 GHz). The set of measurements for identifying paramagnetic metals complexed with HA was performed at a temperature (T) of $-160\,^{\circ}$C, using a magnetic field centered at $(H_o) = 0.26$ T, frequency of microwave (ν) = 9.44 GHz, spectral window of 0.5 T, modulation amplitude (MA) = 1 mT peak to peak, microwave power (P) = 10 mW. Saturation curves were obtained by scanning the magnetic field centered at $H_o = 0.34$ T, with spectral window of 7 mT, ν = 9.44 GHz, MA = 0.1 mT peak to peak and changing the microwave power from 200 nW to 200 mW at room temperature.

An additional set of analysis for detecting SFR at room temperature was made under the following experimental conditions: $H_o = 0.34$ T, ν = 9.44 GHz, spectral window of 7 mT, MA = 0.1 mT peak to peak, P = 0.2 mW. The absolute concentration of free radicals signals was measured using a ruby crystal as secondary standard (Martin-Neto et al., 1991) calibrated with Varian strong

pitch. The area of EPR signals was calculated by double integration of the first-derivative of the absorption signal.

3. Results and discussion

The complete EPR spectrum of the HA sample extracted from treatment N2, which was measured at $-160\,^{\circ}$C with the magnetic field centered at 0.26 T, is shown in Fig. 1a. The spectrum obtained under the same conditions, but scanning the magnetic field over 0.2–0.4 T using a modulation amplitude of 0.4 mT to allow a better resonance line resolution, is shown in Fig. 1b. Spectra obtained for the other HA samples were similar.

The EPR spectra show resonance lines at the g-values and with the hyperfine constants (A) listed below (Senesi, 1990; Martin-Neto et al., 1991; McBride, 1978; Senesi et al., 1987; Olk et al., 1999): $g = 8.66$ and $g = 4.27$ are ascribed to high-spin Fe^{3+} ions held in inner-sphere complexes in either octahedral or tetrahedral sites with rhombic symmetry; $g = 2.003$ due to SFR; $g = 2.12$ associated with spin–spin interactions among various surface-associated paramagnetic ions, also assigned preferentially to Fe^{3+} ions complexed with HAs in octahedral coordination sites (interstitial iron more available and active); $g_{\perp} = 1.98$ and $A_{\perp} \approx 7.1$–7.3 mT assigned to VO^{2+} complexed with oxygen ligands with axial symmetry.

The identification of VO^{2+} ions was confirmed by spectra simulation using the WIN-EPR SimFonia Program, Version 1.25 (Weber, 1995). First, the broad line associated with Fe^{3+} ions at $g = 2.12$ was simulated and subtracted. The same process was then carried out with the line associated with SFR, and finally an anisotropic eight-line spectrum of the axial type distinctive of VO^{2+} (Fig. 2a) was obtained. Comparison of the experimental spectrum with the simulated VO^{2+} spectrum, using $g_{\parallel} = 1.95$; $A_{\parallel} = 0.0191$ T; $g_{\perp} = 1.96$; $A_{\perp} = 7.3$ mT and width line 2.5 mT, revealed similarity between the spectra (Fig. 2b).

Non-homogeneous power saturation curves are shown in Fig. 3. The optimum power saturation value
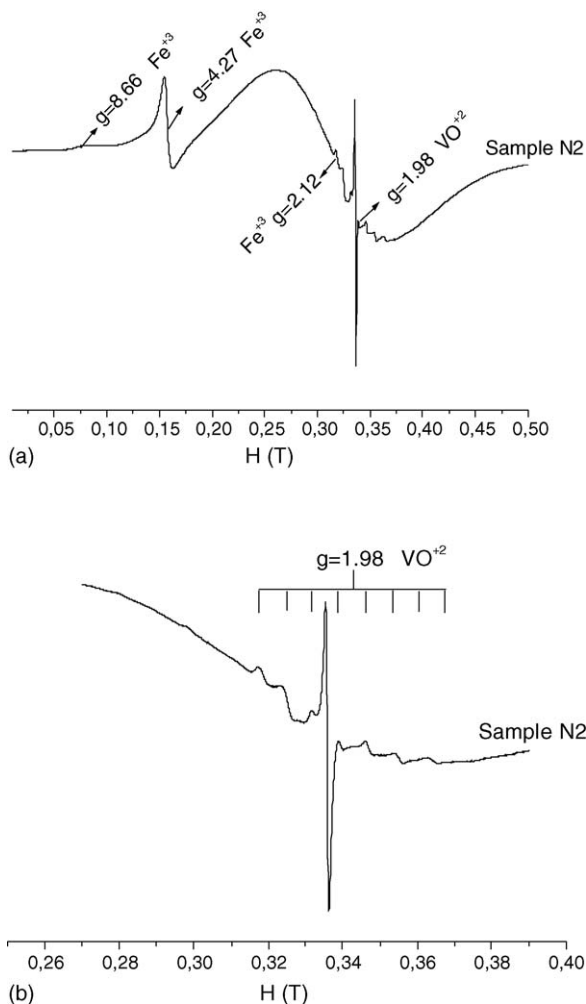
Fig. 1. EPR spectrum of HA (sample N2). (a) Experimental conditions: $\nu = 9.44$ GHz, $P = 10$ mW, $T = -160\,^{\circ}$C, MA = 0.1 mT, $H_o = 0.26$ T and spectral window of 0.5 T and (b) experimental conditions: $\nu = 9.44$ GHz, $P = 10$ mW, $T = -160\,^{\circ}$C, MA = 0.4 mT, scanning magnetic field between 0.2 and 0.4 T.

Fig. 2. (a) EPR spectra of HA (sample NPK) showing: experimental, simulated Fe^{3+}, simulated semiquinone-type free radical and VO^{2+} spectrum obtained after subtraction and (b) comparison between simulated spectrum of VO^{2+} and spectrum of VO^{2+} obtained after subtraction of simulated spectra of Fe^{3+} and SFR from experimental spectrum.

to obtain maximum signal intensity without overlapping or resonance lines saturation was found at 0.2 mW.

The organic free radicals of humic substances at a g-value of 2.0030 are consistent with semiquinone radical units possibly conjugated to aromatic rings, although contribution from methoxy benzene and nitrogen-associated radicals cannot be excluded (Senesi, 1990). The EPR spectrum of SFR from the NPK treatment sample, which is representative of all HA samples, is shown in Fig. 4a.

The three principal EPR parameters, concentration of free radicals, width of resonance line and spectroscopic g-value were determined from SFR spe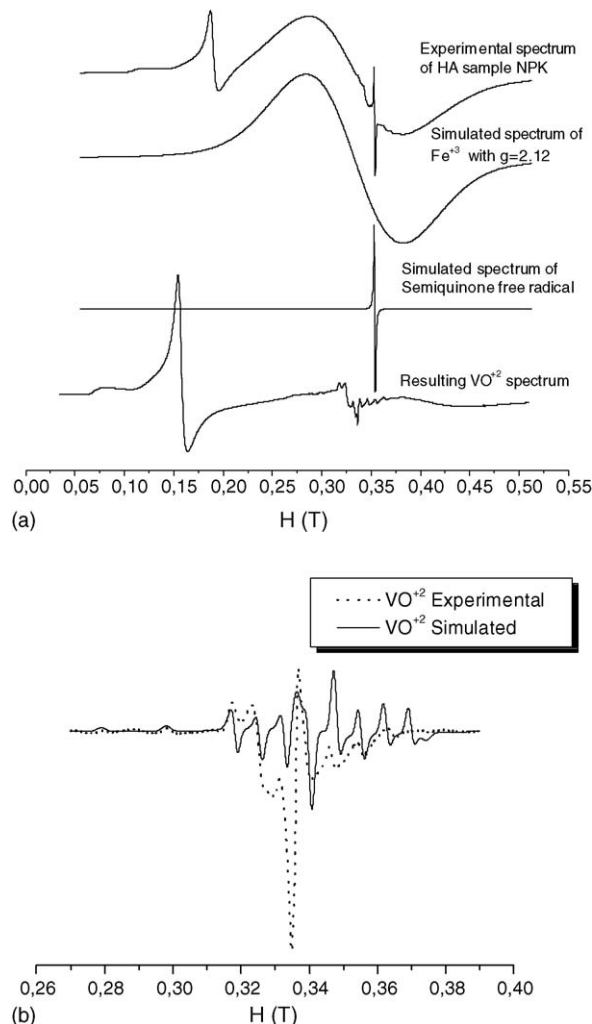ctra. Spin concentration, which is related to concentrations of free radicals in the samples, is shown in Fig. 4b. The width of resonance line was between 4.16–4.52 G and the spectroscopic g-value of 2.0030 for all HA samples. The content of SFR determined from the EPR spectra was approximately 2.0×10^{18} spins g^{-1} for HA from treatments Control, NPK, N1 and N2. The contents of SFR determined from the EPR spectra were lower for treatments N4 and N8, in which the highest application rates of sewage sludge were used (1.7×10^{18} and 1.24×10^{18} spins g^{-1} HA, respectively) (Fig. 4b). This decrease in SFR concentration can be attributed to the incorporation of less aromatic components into organic matter originating from sewage sludge applications.
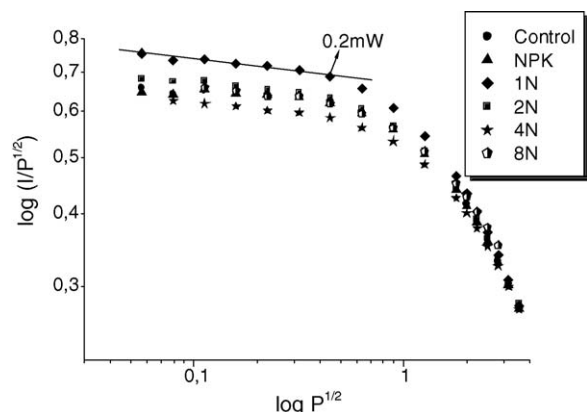
Fig. 3. Power saturation curves for HA samples. Experimental conditions: $H_o = 0.34$ T, spectral window of 7 mT, $\nu = 9.44$ GHz, $P = 200$ nW to 200 mW, $T = 25\ ^\circ$C, MA = 0.1 mT.

In a greenhouse experiment, application of increasing doses of sewage sludge during 4 years, on a sandy loam soil increased the HA content, molecular sizes,
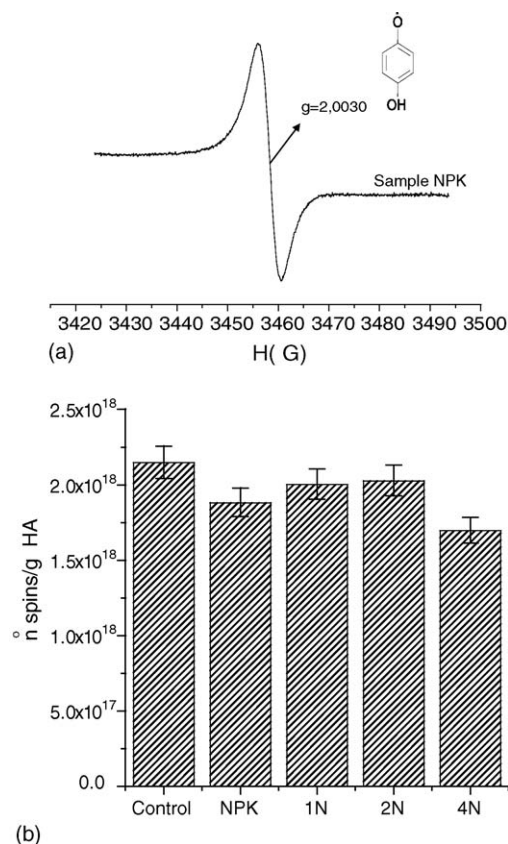

Fig. 4. (a) EPR spectrum of semiquinone-type free radical (SFR) of HA (sample NPK). (b) SFR concentration (in spins g^{-1} HA) determined by EPR for HA extracted from different treatments. Experimental conditions: $H_o = 0.34$ T, spectral window of 7 mT, $\nu = 9.44$ GHz, $P = 0.2$ mW, $T = 25\ ^\circ$C, MA = 0.1 mT.

degree of humification and cation exchange capacity (CEC), and decreased total acidity, COOH content, C/N and E_4/E_6 ratios (Piccolo et al., 1992).

Higher SFR levels and degree of humification, lower C/N ratios and carboxylic group contents were found in HAs extracted from a Brazilian Oxisol amended with sewage sludge (Marques, 1998).

Both of these authors, Piccolo et al. (1992) and Marques (1998), remarked on the improvement in the chemical and physical properties of soil resulting from sewage sludge applications.

Nevertheless, increases in HA contents and decreased C/N, decreased SFR contents and lower degree of humification with increasing sewage sludge doses were measured in a Domino clay loam soil, after applications of sewage sludge over a period of 4–7 years (Senesi et al., 1989). These authors attributed the decrease in SFR contents to the incorporation of components of the applied sludge organic fractions into the soil HAs, i.e., proteinaceous materials and phenolic compounds.

The present results show a reduction in C/N ratios (Table 1) and SFR concentrations (Fig. 4b) with increasing sewage sludge doses ($r = 0.67$ $P < 0.1$), in accordance with the results of Senesi et al. (1989).

Furthermore, the effects of sewage sludge applications may be associated with other factors, such as climatic conditions, soil origin and genesis, as well as the kind of organic wastes used and the composting methods.

Mahieu et al. (1999) reported that the processes controlling decomposition of plant materials added to the soils are essentially the same in temperate and tropical regions, and that the rate of decomposition varies depending on temperature and humidity. Thus, the OM decomposition is faster in tropical climates than in temperate zones.

Bayer et al. (2001) showed that clay minerals and iron oxides determine the physical protection of soil organic matter, and that this physical stability is caused by interaction with variable-charge minerals.

As shown in the present study, the application of organic amendments to soil allows the incorporation of humic substances into the soil OM. There is retention of the carbon in the soil and this in turn, affects soil properties, resulting in increased pH and CEC, an enhanced role of humic substances in contributing to the stabilization of aggregates, avoiding degradation and erosion of soil, increased moisture retention, promotion of plant growth, and interaction with anthropogenically derived organic chemicals in soil and water environments.

4. Conclusions

The highest doses of sewage sludge applied resulted in a decrease in SFR concentration, as determined from the EPR spectra. Considering that this parameter is related to the degree of humification, its decrease can be attributed to lower degrees of humification associated with incorporation of less aromatic components into OM, originating from sewage sludge applications. This indicates that even in a tropical climate microorganisms were not able to decompose the large amount of sewage sludge (N8) incorporated in the soil after 2 years.

Acknowledgements

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