Article

Electron Paramagnetic Resonance Characterization of a Humic Acid-Type Polymer Model

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Modelos de ácidos húmicos do tipo poliméricos tem mostrado semelhanças químicas com os ácidos húmicos naturais (AH). Devido à complexidade das substâncias húmicas (SH), esses modelos de ácidos húmicos do tipo poliméricos, no qual os sítios ligantes podem ser melhores definidos, podem ser usados no estudo de metais e outras aplicações. Neste estudo, obteve-se um polímero com características análogas às dos AH coletados em área de cultivo com sistema plantio direto por 9 anos, por meio de reações de síntese, através da polimerização oxidativa em meio alcalino utilizando como precursor a *para*-benzoquinona. Foi realizado um estudo do ácido húmico (AH) e do ácido húmico sintético (AHS) utilizando espectroscopia de ressonância paramagnética eletrônica (RPE). O sinal dos radicais livres do tipo semiquinona foi detectado para o AHS. Nos espectros observam-se sinais semelhantes entre o AH de solo e o AH sintético complexado com íons Fe^{3+}, Cu^{2+} e Mn^{2+}.

Humic acid-type polymer models have been shown to be similar to natural humic acids (HA) in terms of chemical properties. Due to the complexity of humic substances (HS), this humic acid-type polymer models, in which the ligand sites are better defined, can be used in the study of metals and for other applications. In this study, a polymer with characteristics analogous to those of humic acids isolated from soil surface cultivated using a no-tillage system for nine years, was obtained by means of synthesis reactions, through oxidative polymerization in an alkaline medium using *para*-benzoquinone as precursor. An EPR spectroscopy study was made of natural humic acids (HA) and synthetic humic acids (SHA). A semiquinone-type free radical signal was detected in the SHA. The spectra showed similar signals for HA from soil and synthetic HA complexed with Fe^{3+}, Cu^{2+} and Mn^{2+} ions.

Keywords: synthetic humic acid, metal cations, EPR

Introduction

Humic substances (HS) are well known for being the major component of soil organic matter (SOM) and for their ability to interact with various elements of the environment. HS play a significant role in soil and aquatic chemistry.

HS constitute almost all the organic matter of soil, and due to their high reactivity, they are the fraction involved in most of the chemical reactions that occur in soil. The chemistry and structure of HS are highly complex, which is why their molecular formula is not well defined.[1] Depending on the source of organic material involved in the humification process, HS have a variety of molecular structures that differ according to their source. Analytical limitations and complex and dynamic nature of SOM

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make difficult its characterization. To obtain further information about the chemical structure of the HS, synthetic compounds with polymeric chains have been used whose characteristics are analogous to those of HS, such as phenolic polymers.[2-6] Zunino *et al.*[7] used models of polymeric-type humic and fulvic acids to study the competitive behavior of Zn^{2+} and Mg^{2+}. Significant progress in the study of metals can be achieved using polymers as models similar to HS, in which the ligand sites are better defined, since functional groups and electron donor atoms can be defined using specific precursors.

The literature contains several examples of the application of electron paramagnetic resonance (EPR) in the study of HS.[8-10] EPR spectroscopy is one of the only techniques that can supply structural information, without artifacts or restrictive experimental conditions, about the complexation of metallic ions (Fe^{3+}, Cu^{2+}, Mn^{2+}, VO^{3+}, Cr^{3+} and others) with HS from soils, water and sediments.[11] EPR can also detect free radicals in HS, determining their concentration and sometimes their origin. HS contain stabilized free radicals in their structure, and it has been proposed that they may be associated with polymerization/depolymerization reactions, and with the degree of aromaticity and humification of organic matter, possibly interacting with pesticides and toxic organic pollutants.[11]

MacCarthy[12] postulated that humic substances are unique in nature and have qualities unlike any other natural substances. The first principle postulates that humic substances represent a supermixture of an extremely wide array of chemical structures. Thus, the probability of finding two identical structures is exceedingly remote.[13] Humic substances, of which humic acid (HA, insoluble at acidic pH) and fulvic acid (FA, water-soluble at acidic to alkaline pH) are the major fractions, present various chemically reactive functional groups, including carboxyls, and phenolic and alcoholic hydroxyls with pH-dependent properties.[14] This diversity of their chemical composition allows HS to be used in different situations. Venancio *et al.*[15] demonstrated that the properties of HS can be used in the fabrication of sensor arrays for use in the classification of humic substances present in aqueous systems, as well as the possibility of using HS for the production of self-assembled films. A novel and simple method electrode with rapid preparation was developed with humic acid and polypyrrole. The modified electrode displayed good and reversible extraction properties.[16] Some specific humic molecules play an important role in industrial and pharmacological applications, such as the production and use of therapeutic chemicals from humic acids and the manufacture of commercial humates for use in soils.[17]

Within this context, the objective of this research was to study polymeric models of humic acids, using EPR spectroscopy confronting the results with the HAs extracted from soil under no-tillage system for nine years.

Experimental

Sample preparation

Two different materials were evaluated, a natural humic acid (HA) extracted from a soil, and a compound synthesized (SHA) from *para*-benzoquinone. The soil sample used for the extraction of humic acid (HA) was collected from surface (0 to 25 mm) an area under nine years of management, involving no-tillage system in the cultivation of oats with common vetch/corn with cowpea. The soil type corresponds to a Typic Paleudult by US taxonomy with 540 sand, 240 silt, and 220 g kg^{-1} clay. The clay fraction type is composed mainly of kaolinite (720 g kg^{-1}) and iron oxides (Fe_2O_3, 109 g kg^{-1}).

Humic acid (HA) was extracted from Paleudult soil, following the methodology proposed by the International Humic Substances Society (IHSS).[18]

The polymer (SHA) was prepared with 5.4 g of *para*-benzoquinone dissolved in 60 mL of water containing 2.8 g of KOH. The solutions were placed in flasks and a stream of purified air was bubbled into them at a rate of 1 L min^{-1}. The air was purified by first passing it through solid NaOH pellets, after which it was bubbled in a solution of 0.8 mol L^{-1} NaOH. After 22 h of air bubbling, the resulting black viscous solutions were treated with 6 mL of concentrated hydrochloric acid under mechanical stirring until it precipitated into a brownish black mass. The polymeric products were collected by vacuum filtration, washed repeatedly with deionized water, and dried in air for one day and subsequently at 60 °C for one day.

Preparation of synthetic humic acids (SHA) metal complexes

Complexes of the polymeric SHA with the Cu^{2+}, Fe^{3+} and Mn^{2+} ions were prepared by adding 0.1 g of each polymer to 40 mL of a 0.10 mol L^{-1} solution of each metal sulfate. The suspensions were shaken for 16 h. The samples were centrifuged at 2,200 rpm for 20 min and the supernatant discarded. Deionized water (10 mL) was added, the solids shaken for 2 min and again centrifuged at 2,200 rpm for 20 min, and the supernatant discarded. This washing procedure was repeated twice, after which the purified products were collected and freeze-dried.

Elemental analysis and determination of the ash contents

The elemental composition of the resulting humic acids was characterized based on the quantification of nitrogen, carbon, hydrogen and sulfur, using a CHNS-O analyzer (CE-Instruments EAGER 200), and determination of the difference in percentage by mass of oxygen: $\%O = 100 - [(C + H + N + S)\%]$.

The ash content was determined gravimetrically by weighing and calcining approximately 10 mg of humic acid (HA and SHA) in a platinum crucible at a temperature of 600 °C until it reached a constant mass.

EPR analyse

The experiments were carried out with a Bruker EMX EPR spectrometer operating at X-band (9 GHz), with a frequency modulation of 100 KHz, using a rectangular cavity. The samples were placed in a 3.5 mm diameter quartz tube, in duplicate, with a sample height of 50 mm and mass varying from 10 to 30 g. The central field was $H_0 = 340$ mT, $\nu = 9.72$ GHz, and the microwave power was 0.2 mW with a modulation amplitude of 0.2 mT at room temperature. The semiquinone-type free radicals were quantified by the secondary standard method, using a ruby crystal (Al_2O_3) containing 0.5% of Cr^{3+} *per* weight.[19] Its *g* factor is 1.9797 and it therefore does not interfere with the signal of the semiquinone free radical, shoes *g* values is around 2.003.[20]

To identify the metal ions complexed with HA and SHA, measurements were taken using the following parameters: for the Fe^{3+}, the central field was $H_0 = 286$ mT, scan rate $\Delta H = 400$ mT, frequency $\nu = 9.453$ GHz, microwave power = 10 mW, and modulation amplitude = 1.0 mT at room temperature. The same parameters were used for Cu^{2+} and Mn^{2+}, except that the scan rate was 200 mT at room temperature and at the temperature of liquid nitrogen.

Results and Discussion

Obtaining natural and synthetic humic acids

In the extraction of humic acid from the soil, 710.5 mg of humic acid *per* 0.08 kg of soil were obtained.

It is known that polyhydroxy phenols, as well as hydroquinones and quinones such as *para*-benzoquinone, are highly sensitive to the action of aqueous alkali solution, yielding polymeric products known as synthetic humic acids (SHA).[21] The reason for synthesizing these polymers was that the information obtained from these synthetic precursors can be used to shed light on the complex structure of natural humic acids.
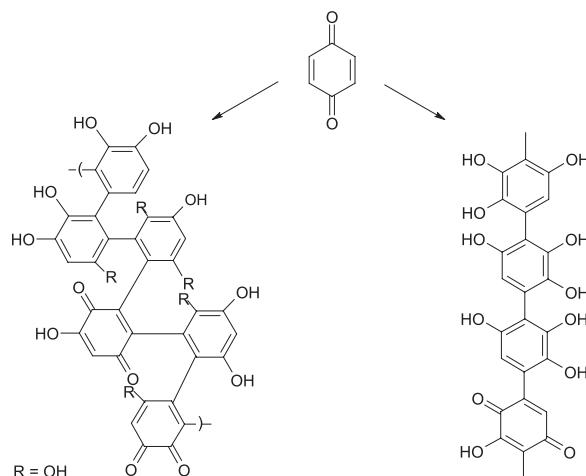

Figure 1. SHA obtained by oxidative polymerization in alkali.

The SHA polymer formed from the *para*-benzoquinone reaction provides a compound composed mostly of polyhydroxy phenols, in which the main adsorbing groups are -OH and -COOH. This structure can be described by a sequence of phenyl rings having three hydroxyl groups *per* ring intercalated by monohydroxylated quinone units (Figure 1).[21] The presence of the carboxylic group is derived from the degradation of the aromatic ring during polymerization. This structure is similar to lignin, one of the major constituents of the organic matter of soil.[21]

The SHA synthesis presented an excellent performance, yielding 76.36% in weight of polymerized *para*-benzoquinone.

Elemental analysis and determination of ash content

Table 1 lists the results of the elemental analysis and the ash content, indicating that the SHA contained a large amount of oxygen exceeding 40% (values calculated by difference), which is similar to the amount of oxygen found in natural humic acid, which also contains more than 40% of oxygen. According to Schnitzer and Kahn,[22] the elemental composition of humic acids contains average amounts of 53.8-58.7% of carbon, 3.2-6.2% of hydrogen, 32.8-38.3% of oxygen, 0.8-4.3% of nitrogen and 0.1-1.5% of sulfur. These values are similar to the ones obtained from the HA extraction. The synthesized polymer differed only in the values described by Schnitzer and Kahn for sulfur and nitrogen content of SHA, which was expected due to the precursor compounds.

EPR study

Figure 2 shows the EPR spectra of the SHA and HA samples. These signals are typical of organic free

Table 1. Elemental compositions of humic acid isolated from soil (HA) and synthetic humic acids (SHA) (given as percentages of ash-free substance basis)

Samples	N	C	H	S	O*	Ash content % (m/m)
			%			
HA	3.90 ± 0.01	44.50 ± 0.01	4.91 ± 0.01	0.71 ± 0.02	45.99 ± 0.02	7.0
SHA	0.30 ± 0.01	55.61 ± 0.02	3.40 ± 0.01	0	40.69 ± 0.02	0.8

*Calculated by difference: %O = 100 – [(C + H + N + S)%]

radicals.[23] Table 2 lists the values obtained for the number of spins g^{-1} HA of free radicals, the values of *g*, and the spectral linewidth. The *g* factor provides information about the chemical nature of the species that contains the unpaired electron, in the case of free radicals. Note the sloping baseline in the spectrum of the soil HA sample, which is due to overlapping of the signal (broadening) of Fe^{3+} at *g* = 2, as indicated in Figure 4.
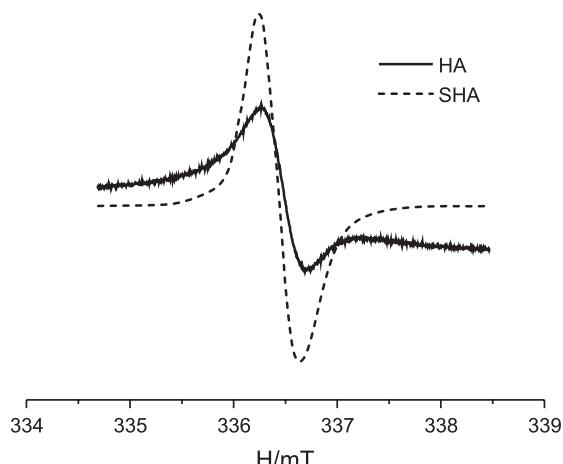

Figure 2. EPR spectrum of the humic acid (HA) sample extracted from soil, and of the sample synthesized from *para*-benzoquinone (SHA without metals), showing the free radical signal.

Table 2 indicates that the linewidth ranged from 0.395 to 0.418 mT and the calculated *g* factor was *ca.* 2.003, thus demonstrating that the paramagnetic resonance of the species, both in the humic acid (HA) sample extracted from soil and in the synthetic humic acid sample, has the same origin, *i.e.*, it is the semiquinone free radical.[24] A comparison of the spectra in Figure 2 indicates a similarity between the HA spectrum and that of the SHA. Thus, the present data clearly shows that SHA can serve as a good model of the radical of the HA. There was a higher concentration of spins in SHA than in HA, but this apparently lower degree of humification of the sample of natural humic acid may be due to suppression of the EPR signal by paramagnetic ions.[25] Natural humic acid and SHA probably contain more aromatic and carboxylic groups. According to the literature, the content of carboxylic and aromatic groups increases as the humification process advances,[26,27] because these groups

Table 2. Organic free radical content, linewidth, and *g* factor for humic acid (HA) from soil and synthetic humic acid (SHA)

Sample	No. of spins g^{-1} HA, SHA	Linewidth (mT) (HA, SHA)	*g*
HA	2.39×10^{17}	0.418	2.0032
SHA	8.72×10^{18}	0.395	2.0035

are constituents of lignin, whose content diminishes with the transformations of organic matter in soil.[27]

Study of synthetic humic acid with complexed metals

The complexation of copper ions with SHA is favored because the structure obtained for the synthetic humic acid prepared from the *p*-benzoquinone precursor has a predominance of oxygenated groups. In the spectrum measured for SHA complexed with copper, we found that these can be formated of copper-oxygen complex. The value of $g_{//}$ decreases as the number of N atoms increases, instead of oxygen, coordinated in the equatorial position, and the value of $A_{//}$ increases.[9,25] Martin-Neto *et al.*[28] found a Cu^{2+} complex with an axial symmetry, with *g* = 2.28 and A = 17 mT, in which the copper was coordinated with nitrogen atoms. Figure 3 shows the spectrum of the SHA samples complexed with Cu^{2+}, showing a value of $g_{//}$ = 2.32 and $A_{//}$ = 14.3 mT, which are similar parameters as those
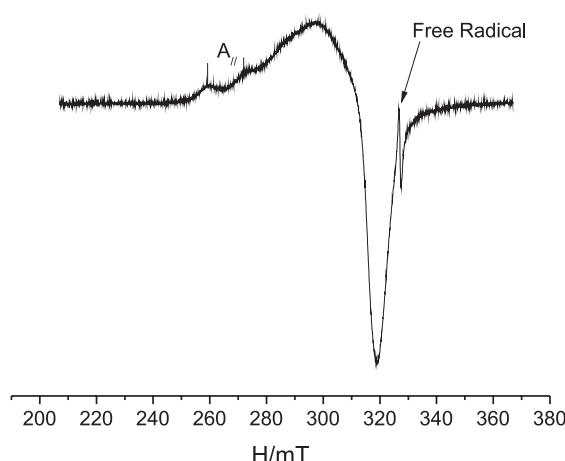

Figure 3. EPR spectrum of the SHA sample complexed with copper: T = 90 K and $A_{//}$ is hyperfine coupling.

found by Boyd *et al.*,[29] Senesi *et al.*[30] and Senesi *et al.*[9] for complexes of humic substances with Cu^{2+} coordinated with oxygen.

Figure 4 shows the spectrum of HA from soil (4a) and SHA complexed with Fe^{3+} (4b) in the region of the Fe^{3+} signals, indicating the values of $g = 4.3$ and $g = 2$. The $g = 4.3$ is due to Fe^{3+} with a rhombic symmetry, while $g = 2$ is probably due to the Fe^{3+} ion in the form of oxide.[31] Note that the HA from soil and the synthetic HA with Fe^{3+} ions show similar signals, indicating that in the soil HAs can be happening the same types of retention of iron ions and native HAs.
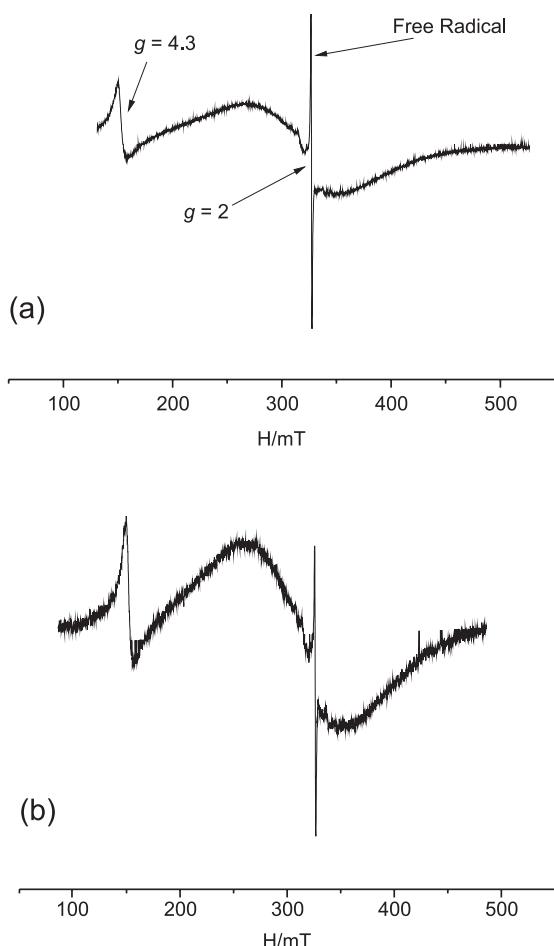

Figure 4. EPR spectrum of (a) the HA sample extracted from soil, and (b) SHA with iron.

Figure 5 depicts the EPR signal of the Mn^{2+} ion complexed in the SHA with hyperfine $A = 8.7$ mT. The EPR spectrum of Mn^{2+} is readily recognized in humic acids by its six hyperfine lines centered around $g = 2$. Mn^{2+} signals are not usually found in purified HA from soil.[32] Values of A between 8.4 and 8.8 mT to HA indicate complex in inner sphere and the functional groups possibly involved they would be carboxylic, hydroxyls phenolic and carbonyl.[22]
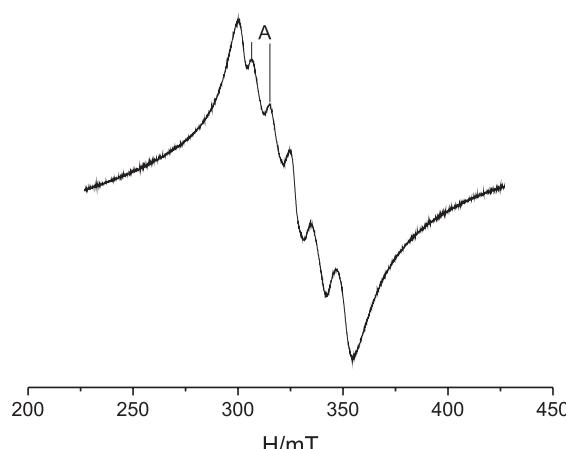

Figure 5. EPR spectrum of the SHA sample with manganese.

Conclusions

In this work, using the technique of EPR, the humic acid produced at laboratory with polymeric of *para*-benzoquinone, structural likeness was shown with humic acid from soil. The sign of the free radical was identified in SHA and with parameters similar to the found in the sign of soil HA. When complexed SHA with metals Fe^{3+}, Cu^{2+} and Mn^{2+} identified characteristic signs of these metals when accomplished measures with soil HA.

Acknowledgments

References

1. Stevenson, F. J.; *Humus Chemistry. Genesis, Composition, Reactions,* John Wiley: New York, 1994.

2. Cosovic, B.; Vojvodic, V.; Boskovic, N.; Plavsic, M.; Lee, C.; *Org. Geochem.* **2010**, *41*, 2000.

3. Giannakopoulos, E.; Drosos, M.; Deligiannakis, Y.; *J. Colloid Interface Sci.* **2009**, *336*, 59.

4. Wang, M. C.; Huang, P. M.; *Geoderma* **2003**, *112*, 31.

5. Wunderwald, U.; Kreisel, G.; Braun, M.; Schulz, M.; *Appl. Microbiol. Biotechnol.* **2000**, *53*, 441.

6. Hanninen, K. I.; Klöcking, R.; Helbig, B.; *Sci. Total Environ.* **1987**, *62*, 201.

7. Zunino, H.; Aguilera, M.; Caiozzi, M.; Peirano, P.; Borie, F.; Martin, J. P.; *Soil Sci.* **1979**, *128*, 257.

8. Rosa, A. H.; Simões, M. L.; de Oliveira, L. C.; Rocha, J. C.; Martin-Neto, L.; Milori, D. M. B. P.; *Geoderma* **2005**, *127*, 1.

9. Senesi, N.; *Adv. Soil Sci.* **1990**, *14*, 77.

10. Czechowiski, F.; Iwona, G.; Jezierski, A.; *Spectrochim. Acta, Part A* **2004**, *60*, 1387.

11. Martin-Neto, L.; Vaz, C. M. P.; Crestana, S.; *Instrumentação Avançada em Ciência do Solo*, Embrapa Instrumentação Agropecuária: São Carlos, 2007.

12. MacCarthy, P. In *Soil Sampling and Methods of Analisys*; Carter, M. R.; Gregorich, E. G., eds.; Canadian Society of Soil Science, CRC Press: USA, 2008.

13. Anderson, D. W.; Schoenau, J. J. In *Soil Sampling and Methods of Analisys*; Carter, M. R.; Gregorich, E. G., eds.; Canadian Society of Soil Science, CRC Press: USA, 2008.

14. Stevenson, F. J.; *Humus Chemistry: Genesis, Composition, Reactions,* Wiley: New York, 1982.

15. Venancio, E. C.; Consolin Filho, N.; Constantino, C. J. L.; Martin-Neto, L.; Mattoso, L. H. C.; *J. Braz. Chem. Soc.* **2005**, *16*, 24.

16. Antilén, M.; Armijo, F.; *J. Appl. Polym. Sci.* **2009**, *113*, 3619.

17. MacCarthy, P.; Rice, J. A. In *Humic Substances in the Global Environment and Implications on Human Health*; Senesi, N., Eds.; Elsevier: Amsterdam, 1994.

18. http://ihss.gatech.edu/ihss2/soilhafa.html, accessed in March 2010.

19. Singer, L.S; *J. Appl. Phys.* **1959**, *30*, 1463.

20. Martin-Neto, L.; Rosell, R.; Sposito, G.; *Geoderma* **1998**, *81*, 305.

21. Cataldo, F.; *Polym. Int.* **1998**, *46*, 263.

22. Schnitzer, M.; Kahn, S. U.; *Soil Organic Matter,* Elsevier Scientific: Amsterdam, 1978.

23. Saab, S. C.; Martin-Neto, L.; *Quim. Nova* **2003**, *26*, 497.

24. Saab, S. C.; Martin-Neto, L.; *J. Braz. Chem. Soc.* **2004**, *15*, 34.

25. Novotny, E. H.; Martin-Neto, L.; *Geoderma* **2002**, *106*, 305.

26. Catroux, G.; Schnitzer, M.; *Soil Sci. Soc. Am. J.* **1987**, *51*, 1200.

27. Zech, W.; Senesi, N.; Guggenberger, K. K.; Lehmann, J.; Miano, T. M.; Miltner, A.; Schroth, G.; *Geoderma* **1997**, *79*, 117.

28. Martin-Neto, L.; Nascimento, O. R; Talamoni, J.; Poppi, N. R.; *Soil Sci.* **1991**, *151*, 369.

29. Boyd, S. A.; Sommers, L. E.; Nelson, D. W.; West, D. X.; *Soil Sci. Soc. Am. J.* **1983**, *47*, 43.

30. Senesi, N.; Sposito, G.; Martin, J. P.; *Sci. Total Environ.* **1986**, *55*, 351.

31. Saab, S. C.; Martin-Neto, L.; *J. Braz. Chem. Soc.* **2008**, *19*, 413.

32. Moreira, S. G.; Prochnow, L. I.; Kiehl, J. C.; Martin-Neto, L.; Pauletti, V.; *R. Bras. Ciênc. Solo* **2006**, *30*, 121.