

Chapter 20

Spectroscopy Characterization of Humic Acids Isolated from Amazonian Dark Earth Soils (*Terra Preta De Índio*)

TJF Cunha, EH Novotny, BE Madari, L Martin-Neto, MO de O Rezende, LP Canelas, and V de M Benites

20.1 Introduction

Soils are one of the most important natural resources, and are essential for the development and continuation of any society that practices agriculture. Ancient civilizations in the Old World generally began in valley regions and floodplains along big rivers. As examples, one can cite Egypt in the Nile River valley, the Mesopotamia between the Tigris and Euphrates rivers, the Indian Subcontinent by the margins of the Indus and Ganges rivers, and China in the valleys of the Yellow and Blue rivers. In different areas of the world, many detailed comparisons have been accomplished between natural and human influenced soils, and the results of the latter have been documented (Cunha 2005)

In the last decades, agricultural activities have been modifying the original vegetation cover of a great part of the Brazilian territory. Ecosystems, such as the Amazon forest, are losing their original characteristics, being replaced by agricultural and extractive activities. The expansion of the agricultural borders has been causing great changes in forest areas in Brazil, with the introduction of rice, soybeans, and pastures, mainly in the southern part of the Amazon area. This has led to degradation and a loss of biodiversity, a reduction in organic matter, and also the degradation of the pedologic covering, through the exhaustion and erosion.

However, in the Brazilian Amazonian area, there are soils with upper horizons of dark coloration that exhibit elevated levels of nutrients, called anthropic A horizons. These soils, even when used for agricultural purposes maintain their fertility characteristics and high contents of organic matter for many years. This epipedon has been found in several types of soils occurring in the Brazilian Amazon, such as Latossols, Argissols, Cambissols, Plintossols, and Nitossols, under forest or agricultural use such as the cultivation of corn, beans, and manioc, in addition to fruit cultivation, horticulture, and even the production of pasture on a wide scale.

The *terra preta* or Amazonian Dark Earths (ADE) are soils that can be distinguished from other soils by particular characteristics that are products of ancient anthropic activities. Most frequently, in the Amazon basin, their formation is attributed to ancient habitation agricultural activities (Woods and McCann 1999) by previous Amazonian civilizations.

Characteristic properties of these soils are the presence of artifacts (e.g. ceramics, lithics, etc.) at any amount, and/or Melich-1-extractable phosphorus (elemental P) in the fine earth fraction (at least 65 mg/kg^{-1}), charcoal in the fine earth fraction, fine earth organic carbon content (10 g kg^{-1} or more) as determined by the Walkley-Black method (Embrapa 1997), in the absence of any other evidence of ancient anthropic activity, the HA: FA ratio must be ≥ 2.0 .

Both the organic C content and the HA:FA ratio should be higher from the soil surface down to the depth of 0.6 m or more in comparison to surrounding background soils. Indicators of anthropic origin can still be the presence of fragments of shells and other aquatic organisms, bone artifacts, or accumulation of organic compost or mud, or solid earth additions with or without cultural artifacts. Clear spatial association of soil with prior human activities can also be indicators of Archaeo-anthropogenic soils. These soils form a wide array of soil classes as indicated by the 4th level units of the proposed Archaeo-pedological Classification (Kämpf et al. 2003).

Today, these soils represent important islands of high soil fertility in the Amazon environment. The exact management practices responsible for the formation of ADE still remain unclear, but the relationships between its organic matter and their production potential have become evident (Cunha 2005).

Several studies were accomplished to understand the genesis of the soils with an anthropic A horizon. However, very little was done in relation to the soil chemical characteristics, physical-chemical, and spectroscopic properties of its organic matter. The study of the humic acids (AH) of the anthropogenic soils, through its spectroscopic characteristic can contribute to the knowledge and the action of the organic matter of these soils, as well as the several aspects related to the structural characteristics, functionality, reactivity, effect of the agricultural use, and its productive potential. Thus, the objective of this work was to study the spectroscopic characteristics of the humic acid of soils with an anthropic A horizon, under agricultural use, compared to others in several areas of the Brazilian Amazon, for the best knowledge of the influence of the organic matter on its micro and macroscopic characteristics.

20.2 Material and Methods

20.2.1 Description and Morphologic Characterization

For this study 18 areas of occurrence of anthropogenic soils were selected in the areas of Humaitá, Lábrea, Apuí, New Aripuanã, Manicoré, and Manaus, in Amazonas State. In those soils, the anthropic A horizon was sampled (Horizon Au1), according to Lemos and Santos (1996). The samples were collected in the 0–20 cm layer, with several sub-samples collected that were mixed and prepared for future analyses. The study areas were selected in function of the different use systems found, embracing areas under forest and cultivated areas. Four soils were also selected adjacent to anthropogenic soils under forest. In the total 22 samples were

analyzed and grouped in function of the current use and soil type, as followed: Anthropogenic Soil under Forest (ASF); Cultivated Anthropogenic Soil (CAS); and the control, Non-Anthropogenic Soil Under Forest (NASF).

20.2.1.1 Extraction, Chemical Fractionation and Purification of the Humic Material

The extraction and the division of the humic substances to obtain the humic acids, as well as their purification, were accomplished according to method suggested by the International Society of Humic Substances (IHSS), whose methodological protocol is described in Swift (1996). The humic acids extraction process involved air drying the soils, H⁺-exchanging (0.1 M HCl), and then extracting overnight with NaOH (0.1 M) under N₂. The supernatants were recovered by centrifugation and filtration, and the pH was immediately adjusted to 2 using 6 M HCl. The residues were re-extracted and the supernatants were mixed. The acidified suspension was centrifuged at 5,000 relative centrifugal forces (RCF) for 10 min and the sediment was re-dissolved in 0.1 M KOH in an atmosphere of N₂. Then this solution was made 0.3 M with respect to KCl and the flocculated colloidal particles were recovered by centrifugation at 40,000 RCF for 15 min. The supernatant was acidified to pH ~2 using 6 M HCl and the precipitated HAs were recovered by centrifugation. The HAs were treated twice with 0.5% HF + HCl for 24 h and centrifuged at 5,000 RCF. The treated samples were recovered by centrifugation (5,000 RCF), washed with 200 ml of 0.01 M HCl, centrifuged (5,000 RCF) again, and the precipitated material was dialysed and then freeze-dried.

20.2.1.2 UV-VIs Spectroscopy and E₄/E₆ Relationship

The spectra in the UV-VIs were obtained according to procedures described in Chen et al. (1977) in a 200 to 800 nm wave length, at 25°C, with 1 cm optic way, in solution prepared with 20 mg of humic acid diluted in 1 L of NaHCO₃ 0.05 mol L⁻¹, with pH around 8.0. For the determination of the relationship coefficient E₄/E₆, the absorbency was divided in 465 nm by the obtained in 665 nm.

The $\Delta \log K$ value was adopted in this study, aiming to analyze the optical properties of the humic acids, because it expresses the nature of the humic substances (Yonebayashi and Hattori 1988). The Log K is defined as the difference among the logarithms of the absorbance at 400 nm ($\log E_{400}$) and at 600 nm ($\log E_{600}$) (Kumada 1987).

20.2.1.3 Diffuse-Reflectance Infrared Fourier Transform (DRIFT) Spectroscopy

For the study of the DRIFT, the spectra were obtained in units of Kubelka-Munk, with a nominal resolution of 4 cm⁻¹, inside of the spectral strip between 4,000 and 400 cm⁻¹. The samples of humic acid were analyzed by a spectrophotometer adapted with an unit of diffuse reflectance, being used approximately 5 mg of AH and

250 mg of KBr, that were conditioned in a cell of 1.2 cm of diameter, being the spectra treated in a similar way to the spectra obtained by absorption spectroscopy.

20.2.1.4 Aromaticity, Hydrophobicity and Reactivity Index

The relationship among the absorbance of the absorption band in the 3.057–3.055 cm^{-1} and in 2.934–2.928 cm^{-1} region was called in this work of aromaticity index, once it expresses the relationship between the stretching of C-H of aromatic groups and asymmetric axial stretching of C-H aliphatic, respectively.

On the other hand, the relationship among the absorbancy of the absorption band in the 2.928 cm^{-1} and 1.050–1.080 cm^{-1} region, was called, in this work, of hydrophobicity index, once it expresses the relationship among apolar (CH_3) and polar (-OH, C-O) groups respectively. The reason among the absorption bands for 1.720–1.722/1.525, was called reactivity index, for expressing the relationship among stretchings C = O of COOH and ketones and the stretching of aromatic C = C.

20.2.1.5 Electron Paramagnetic Resonance (EPR)

The study of organic free radicals was accomplished by EPR, through the Bruker-EMX spectrometer with rectangular cavity, operating in band X (approximately 9.0 GHz) under room temperature conditions. The concentration of organic free radicals (spins g^{-1}) was obtained by the integration of the signal of the first derivate of the free radicals, compared to the pattern of strong pitch, supplied by Bruker, with concentration of well-known spins (3×10^{15} spins cm^{-1}), using the synthetic ruby as secondary pattern (Martin-Neto et al. 1994).

The amounts of free radicals were normalized by mass (or carbon content). The line width was estimated through pick by pick separation of the first derivate of the sign absorption (Senesi and Steelink 1989). The measurements of RPE were accomplished in a spherical resonant cavity, with magnetic field (H_0) of 340 mT, modulation frequency of 100 kHz, modulation amplitude of 0.05 mT and 0.2 mW microwave potency.

20.3 Results and Discussion

20.3.1 UV-Visible Spectroscopy Analysis

In all the spectra obtained in the UV-Vis region, a decrease on the absorption intensity with an increase of the wave length was observed. A discreet “shoulder” was observed around 270 nm (Fig. 20.1). This is possibly related to the fact of this material possesses chromophores that absorb in the whole analyzed region, as noted by

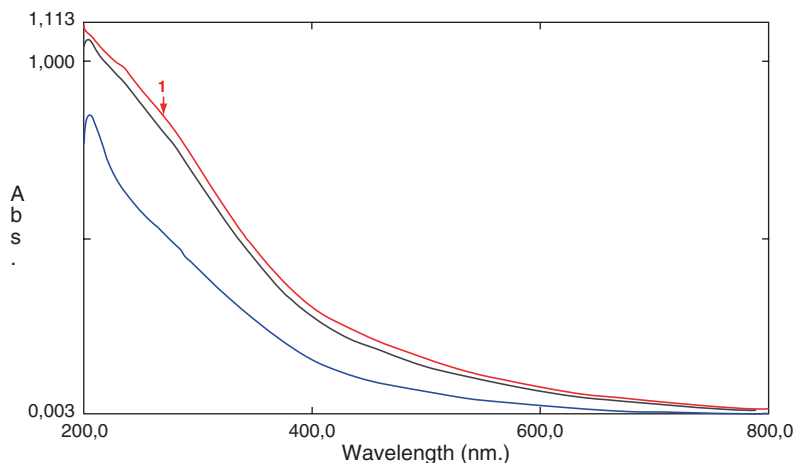


Fig. 20.1 Representative spectra of light absorption in the strip of the ultraviolet and of the visible light of the samples for groups of humic acids. In black, group ASF; in red, group CAS and in blue, group NASF

Stevenson and Schnitzer (1982). This “shoulder” can be attributed to the presence of aromatic structures. Analyzing samples of several origins, Ghosh and Schnitzer (1979) observed a “shoulder” in the region of 250 to 270 nm, and they attributed it to the structural conjugation of quinone and ketones, therefore indicative of aromatic systems (Canellas 1999).

The values of $\Delta \log K$ differed significantly among the groups ASF and CAS and the group NASF, indicating that the humic acids of the groups ASF and CAS, that present low values for $\Delta \log K$ (Fig. 20.1) in relation to the humic acid of the NASF group, possesses high humification degree, and are in agreement with results obtained by Ishiwatari (1985) and Yonebayashi and Hattori (1988).

Using the absorption characteristics in the UV-Vis region, the humic acids can be classified in 4 different types: A, B, Rp, and P (Kumada 1987). Humic acids of the A type do not present characteristic absorption bands and the values for $\Delta \log K$ are smaller than 0.6. Humic acid of the B type presents a weak “shoulder” of absorption at 275 nm and the value of $\Delta \log K$ among 0.6 to 0.8. Humic acids of the Rp type present the same spectrum of the B type, but with elevated values of $\Delta \log K$ (between 0.8 and 1.1). The humic acids that present characteristic absorptions in the 615, 570, and 450 nm visible regions are considered as the P type. Analyzing the $\Delta \log K$ data (Table 20.1), the humic acid of the groups ASF and CAS can be classified as the A type and the humic acid of the group NASF as being of the B type, suggesting that the humic acids of the groups ASF and CAS possess a larger evolution, that is reflected in its smallest relation E_4/E_6 .

The weak radiation absorption in the region of the visible for humic substances is of uncertain cause (Bloom and Leenheer 1989), but it is useful for allowing the obtaining of the reason E_4/E_6 , used as humification parameter. The relationship E_4/E_6 is related to the aromaticity and to the degree of condensation of the chain of

Table 20.1 Differences in the optical properties among the groups of humic acids

Groups	n°	$\Delta \log K$	E_4/E_6
ASF	6	0.5 b	4.2 b
CAS	12	0.5 b	4.2 b
NASF	4	0.7 a	6.0 a

Different letters in the same column differ significantly at 5%

aromatic carbons of the humic acids, and could be used as a humification index (Kononova 1966; Stevenson and Schnitzer 1982). The values obtained in the relationship E_4/E_6 (Fig. 19.1) were located around 4.0 for the humic acids of the groups ASF and CAS and around 6.0 for the humic acids of the group NASF. Kononova (1982) suggests values of the relationship E_4/E_6 for humic acid smaller than 5.0 and between 6.0 and 8.0 for fulvic acids. The humic acids of the group NASF, is inside the strip indicated for fulvic acids, suggesting these humic acids are less chemically evolved in relation to the CAS and ASF groups.

The cultivation did not alter the values of the relation E_4/E_6 among the humic acid of the groups ASF and CAS. In that way, based on Kononova (1966) and Stevenson and Schnitzer (1982), this behavior is indicative that the same did not affect the levels of condensation of the aromatic rings of these humic acids.

20.3.2 DRIFT Spectroscopy Analysis

The absorption spectra in the infrared region presented characteristic absorptions, but with relative variable intensities. In the region below $1,800\text{ cm}^{-1}$, some important differences were observed. The similar characteristics (typical bands of humic acids), but with different relative intensities, are related to an intense and large absorption band centered among $3,470$ and $3,300\text{ cm}^{-1}$ attributed to the stretching of O-H (contribution of OH aliphatic and amina); a pick of medium intensity, centered between $3,061$ and $3,055\text{ cm}^{-1}$, attributed to the stretching of aromatic C-H; a band of intense absorption among $2,934$ – $2,928\text{ cm}^{-1}$ and a weaker absorption band between $2,857$ and $2,853\text{ cm}^{-1}$, attributed to asymmetric and symmetrical stretching of C-H aliphatic, respectively; an absorption band between $1,246$ and $1,250\text{ cm}^{-1}$, attributed to the bending of O-H and stretching of C-O of the carboxylic group and a weak absorption band in the region from $1,080$ to $1,049\text{ cm}^{-1}$, attributed to the stretching of C-O of polysaccharides and silicates sludges.

The different characteristics observed in the area below $1,800\text{ cm}^{-1}$ of the spectra of DRIFT were concentrated in the $1,720$ to $1,709\text{ cm}^{-1}$ region, attributed to the stretching of C = O of carboxylic acids, aldehydes and ketones, whose relative intensities are strong for the humic acids of the group CAS, averages for the humic acids of the group ASF and weak (when existent) for the humic acids of the group NASF. In fact, the relation among the $1,720$ – $1,722/1.525$ absorption bands (denominated

in this work of reactivity index) was of 1.15 and 1.17 for the humic acids of the groups SAF and CAS, respectively. The values above 1.0 indicate a larger relative concentration of stretchings $C = O$ of the carboxylic groups compared to the stretching aromatic $C = C$. The calculation for the group NASF was not possible due to the absence or little definition of those bands.

An absorption band between $1,661$ and $1,628\text{ cm}^{-1}$, observed only for the humic acids of the group NASF, attributed to the stretching $C = O$ of groups amide (band I of the amide), and also $C = O$ of quinones and H tied to conjugated ketones; a fourth peak between $1,614$ and $1,612\text{ cm}^{-1}$, generally attributed to the stretching of aromatic $C = C$ and symmetrical stretching of the anion COO^- , that are only present in the humic acids of the groups ASF and CAS; an absorption in $1,553\text{ cm}^{-1}$, attributed to the N-H deformation of the amide (band II of the amide) and stretching of $C = N$ just observed in the spectra of the humic acids of the NASF group; a band in the region from $1,354$ to $1,327\text{ cm}^{-1}$, attributed to the symmetric deformation of CH_3 , just observed in the spectra of the NASF group; absorption in the region from 762 to 669 cm^{-1} , attributed to the angular deformation out of the plan of connections C-H of aromatic rings, observed in the spectra of the ASF and CAS groups.

In general, the data obtained through the DRIFT spectroscopy, suggest a mixture of aromatic/aliphatic characteristics, a great amount of carboxylic groups and a smaller number of N- containing groups for the humic acids of the ASF and CAS groups. The humic acids of the group NASF was characterized for presenting a larger content of of N- containing components, for example, polypeptide chains (Senesi et al. 2003), and a smaller content of carboxylic and fenolic groups, demonstrating that qualitatively the humic acids of the NASF group are less reactive than the ones of the ASF and CAS groups and that N is a important part of the structures of the humic acids of the NASF group. Among the ASF and CAS groups, the reactivity index was relatively larger for the CAS group (1.17), meaning the humic acids of this group are the more reactives.

20.3.3 Aromaticity (IA) and Hydrophobicity Index (IH)

In Table 20.2 it can be observed the medium values for the aromaticity and hydrophobicity index in the different groups of humic acid. The observed aromaticity index was larger in the anthropogenic soils. The humic acid from the soils under forest (ASF and NASF groups) came out more hydrophilic than the humic acid from cultivated soils (CAS).

Table 20.2 Aromaticity (IA) and hydrophobicity index (IH) obtained of the infra-red spectra

Groups	IA	IH
ASF	0.87 a	0.37 b
CAS	0.85 a	0.48 a
NASF	0.77 b	0.35 b

Different letters in the same column differ significantly at 5%

20.3.4 EPR Spectroscopic Analysis

The spectra were characterized by a unique line of fine and symmetrical absorption without any hyperfine interaction, with the value-g centered around 2.0033 (Fig. 20.2). Among the humic acid extracted from anthropogenic soils, the ones from the CAS group presented the smallest line width, together with the concentration values of organic free radicals (CRLO) indicate that these were more humified (Table 20.3).

The line width value is related with the time that the excited electron spends to return to the environment the absorbed energy (relaxation time) and also with the interaction of the organic free radical with the environment. The more condensed the molecules, the smaller the line width (Senesi and Steelink 1989). The largest value for line width was observed for the humic acids of the NASF group indicating smaller condensation and aromaticity of the humic acid belonging to this group.

The concentration of organic free radicals, in the humic acids of the CAS group, was three times higher than the concentration obtained for the humic acids of the NASF group. Significant differences were also observed in the concentration of organic free radicals of the ASF and CAS groups, suggesting the humic acids of the ASF group are less aromatic than the humic acids of the CAS group. The humic acids of the NASF group would be the less aromatics of all the humic acids studied.

Fig. 20.2 Typical spectra of EPR of samples of humic acids belonging to the studied groups (I = intensity of the sign; ΔH = width of the line). Experimental Conditions: $H_0 = 3,400$ G, $\Delta H = 100$ G = 9.44 ghz, temperature sets, modulation Width = 0.5 G

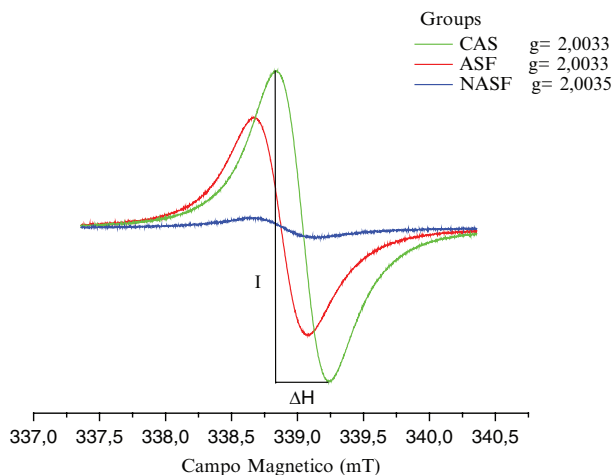


Table 20.3 Data of EPR for groups of humic acids

Groups	CRLO	Line width	
	Spin $g^{-1} \times 10^{17}$	Gauss	Value-g
ASF	4.07 b	4.27 a	2.0033 a
CAS	6.59 a	3.73 b	2.0033 a
NASF	2.11 c	4.32 a	2.0034 a

Different letters in the same column differ significantly at 5%.

20.4 Conclusions

The HA of the anthropogenic soils are different from the HA of the non-anthropogenic soils. The main differences among the ASF and CAS groups, in relation to the NASF group were in the humification degree of HA. The HA belonging to the ASF and CAS groups were in a more advanced stage of humification than those of the NASF group. The largest humification degree of HA of the anthropogenic soils were due to the larger concentration of aromatic structures and of organic free radicals. Some results suggest that this fact is related to the pyrogenic origin of these HA.

The HA of the anthropogenic soils under forest and under cultivation are different. The main differences among HA of the ASF and CAS groups were related to the hydrophobicity. The HA of the CAS group were more hydrophobic.

Also, differences were detected in the stability characteristics of HA of the ASF and CAS groups. The HA of the CAS group had a larger concentration of more aromatic structures which could be due to the selective preservation of these structures in comparison to the HA of the ASF group.

In the anthropogenic soils that are rich in the humified organic matter, recalcitrant and, at the same time, of high reactivity, there is a favorable environment for ionic exchange, reactions with inorganic colloids of the soil, with organic molecules and of complexation with metallic cations, among others. Due to the fact of the anthropogenic soils possessing more humified and stable organic matter than the organic matter of non-anthropogenic soils, these soils probably processes that favor the carbon accumulation and the formation of stable structures.

The high fertility, sustainability, and consequently, the high agricultural potential use of the anthropogenic soils, at least partly can be attributed to the physical-chemical characteristics of the HA fraction of the organic matter of the soils with anthropic A horizon.

The spectroscopic technics of UV-Vis, DRIFT and RPE used in this study were complementary in the obtaining of information on the physical-chemical characteristics of HA, demonstrating the great potential of those tools in the studies of humics substances coming of soils of anthropogenic origin.

Acknowledgment The authors express their thanks to Dr. José Moacir Pinheiro de Lima Filho for translation of this manuscript.

References

- Bloom P Leenheer JA (1989) Vibrational, electronic, and high-energy spectroscopic methods on characterizing humic substances. In: Hayes MHB et al. (ed) Humic Substances II. In Search for the Structure. Chichester: Wiley, pp 409–446
- Canellas LP (1999) Avaliação de características físico-químicas de ácidos húmicos. 164f. Tese (Doutorado) - Universidade Federal Rural do Rio de Janeiro, Seropédica

- Chen Y, Senesi N, Schnitzer M (1977) Information provided on humic substances by E4/E6 rations. *Soil Science Society of America Journal*, Madison, WI, 41:352–358
- Cunha TJF (2005) Ácidos húmicos de Solos escuros da Amazônia: Terra Preta de Índio. 140f. Tese (Doutorado); Universidade Federal do Rio de Janeiro
- EMBRAPA (1997) Centro Nacional de Pesquisa de Solos. Manual de métodos de análise de Solo. 2 ed. rev. atual. Rio de Janeiro, p 212 (EMBRAPA-CNPS. Documentos, 1)
- Ghosh K, Schnitzer M (1979) UV and Visible absorption spectroscopic investigations in relation to macromolecular characteristics of humic substances. *The Journal of Soil Science*, Oxford, 30:735–745
- Ishiwatari R (1985) Geochemistry of humic substances in lake sediments. In: Aiken GR et al. (ed) *Humic substances in soil, sediment, and water: geochemistry, isolation and characterization*. New York: Wiley Interscience Publication
- Kämpf N, Woods WI, Sombroek W, Kern DC, Cunha TJF (2003) Classification of Amazonian dark earth and other ancient athropic soils. In: Lehmann J, Kern DC, Glaser B, Woods WI (2003) *Amazonia Dark Earth: Origin, Properties, Management*, Kluwer Academic Publishers, Dordrecht-Boston-London, pp 77–102
- Kononova MM (1982) *Materia organica del suelo: su naturaleza, propiedades y métodos de investigación*. Barcelona: Oikos-tau, p 364
- Kumada K (1987) *Chemistry of soil organic matter*. Tokyo: Japan Scientific Societies Press, p 241
- Lemos RC, Santos RD (1996) *Manual de descrição e coleta de solo no campo*. 3 ed. Campinas, Sociedade Brasileira de Ciência do Solo, p 84
- Martin-Neto L, Andriulo AE, Tragheta DG (1994) Effects of cultivation on ESR spectra of organic matter from soil fractions of a mollisol. *Soil Science*, Baltimore, 157:365–372
- Senesi N, Steelink C (1989) Application of ESR spectroscopy to the study of humic substances. In: Hayes MHB et al. (ed) *Humic Substances II. In Search for the Structure*. Chichester: Wiley, pp 373–408
- Senesi N, D’Orazio V, Ricca G (2003) Humic acids in the first generation of EUROSOLS. *Geoderma*, Amsterdam, 116(3/4):325–344
- Stevenson FJ, Schnitzer M (1982) Transmission electron microscopy of extracted fulvic and humic acids. *Soil Science*, Baltimore, 133:179–185
- Swift RS (1996) Organic matter characterization. *Methods of soil analysis*. Madison: Soil Science Society of America: American Society of Agronomy, In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) (*Soil Science Society of America Book Series*, 5). Part 3. Chemical Methods, pp 1011–1020
- Woods WI, McCann JM (1999) The anthropogenic origin and persistence of Amazonian Dark Earths. In: Caviedes C (ed) *Yearbook 1999 – Conference of Latin Americanist Geographers*, Austin: University of Texas Press, pp 7–14
- Yonebayashi K, Hattori T (1988) Chemical and biological studies on environmental humic acids. I. Composition of elemental and Functional Groups of Humic Acids. *Soil Science and Plant Nutrition*, Tokyo, 34(4):571–584