STRUCTURAL FEATURES OF HUMIC ACIDS ISOLATED FROM SOILS AMENDED WITH URBAN ORGANIC RESIDUES: AN ELEMENTAL COMPOSITION, ^{13}C NMR AND Py-GC/MS ANALYSIS(1)

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SUMMARY

The structural features of humic acids (HA) after addition of organic matter from urban residues were evaluated. The HAs were extracted from the surface (0-20 cm) of an ultisol (Seropédica, RJ) and oxisol (Piraí, RJ) which had been treated with the equivalent of 80 t ha^{-1} (dry material) of compost from urban waste and also sewage sludge. The incubation time in the laboratory was 24 weeks under room temperature and humidity at field capacity. Tha HAs were characterized by elemental analysis, ^{13}C NMR spectroscopy and pyrolysis coupled to gas chromatography/mass spectrometry (Py-GC/MS). NMR spectroscopy allowed the clear observation of alterations in the molecular composition of the HAs, mainly through the increase in the content of mono and dioxygenated carbon atoms (δ 50-100) and decrease in the content of CH_2 and CH_3 groups (δ 0-40). The increase in the amount of carbohydrates suggests the presence of more labile structures in the HAs with organic residues amendment. In addition, Py-GC/MS led to the observation of significant qualitative differences between the compounds which arose from the thermal fragmentation of HAs, mainly lipids and carbohydrate derivatives (such as furan), for soil samples treated with either organic residues. The coupling of ^{13}C NMR spectroscopy and Py-GC/MS has proven useful in evaluating physical changes in HAs isolated from soils treated with urban organic residues.

Index terms: humic substances, soil quality, spectroscopic methods, urban compost, sewage sludge.

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RESUMO: *CARACTERÍSTICAS ESTRUTURAIS DE ÁCIDOS HÚMICOS ISOLADOS DE SOLOS COM ADIÇÃO DE RESÍDUOS ORGÂNICOS DE ORIGEM URBANA: COMPOSIÇÃO ELEMENTAR, ANÁLISE DE RMN ^{13}C E Pi-CG/EM*

Foram avaliadas as alterações nas características estruturais de ácidos húmicos (AH) decorrentes da adição de matéria orgânica de resíduos urbanos. Os AH foram extraídos da camada superficial (0-20 cm) de um Argissolo Vermelho-Amarelo (Seropédica, RJ) e de um Latossolo Vermelho Amarelo (Piraí, RJ), ambos tratados com o equivalente a 80 t ha^{-1} (em base seca) de composto de lixo urbano e lodo de estação de tratamento de esgotos. O tempo de incubação em laboratório foi de 24 semanas à temperatura ambiente e umidade mantida na capacidade de campo. Para caracterizar os AH, foram utilizadas a análise elementar, a ressonância magnética nuclear (RMN) de ^{13}C e Pirólise acoplada à cromatografia gasosa e a espectrometria de massas (Pi-CG/EM). A análise de RMN ^{13}C permitiu observar alterações na composição molecular dos AH de forma clara, principalmente, pelo aumento do teor de C mono e di oxigenados (δ_C 50-110) e pela diminuição dos teores de grupos CH_2 e CH_3 (δ_C 50-110). O aumento no teor de carboidratos evidencia a presença de estruturas mais lábeis nos AH com adição dos resíduos. Em adição, a técnica de Pi-CG/EM permitiu verificar diferenças qualitativas significativas nos compostos orgânicos, provenientes da fragmentação térmica dos AH com incorporação de novas estruturas, sobretudo da fração lipídica e de derivados de carboidratos (furanos), nas amostras de solos tratados com ambos os resíduos orgânicos. O uso em conjunto das técnicas analíticas de RMN ^{13}C e de Pi-CG/EM foi eficiente para avaliar as mudanças na composição molecular de ácidos húmicos decorrentes da adição de resíduos orgânicos de origem urbana.

Termos de indexação: substâncias húmicas, qualidade do solo, métodos espectroscópicos, composto de lixo, lodo de esgoto.

INTRODUCTION

Preservation of soil quality requires maintenance of both quantity and quality of soil organic matter because of its importance in improving soil structure, fertility and biological activity. Humic substances comprise the highest proportion of soil organic matter and arise from plants and animal tissues through chemical transformation as well as biological degradation by microorganisms, resulting in complex chemical structures which are more stable than the original material (Santos & Camargo, 1999). The study of the molecular structure of humic substances has been one the most difficult aspects in the characterization of soil organic matter, due to their complex and irregular structure (Hayes et al., 1996). Spectroscopic methods are the best for assessing changes in the quality of soil organic matter (Beudert et al., 1989; Hempfling & Shulten, 1990; Zech et al., 1992; Preston et al., 1994; Ceretta et al., 1999). ^{13}C-NMR spectroscopy has provided useful information on C-distribution of organic matter due to different soil managements (Preston, 1996; Bayer et al., 2000). The analysis of humic substances by ^{13}C NMR spectroscopy reveals that the fulvic acids (FA) are constituted mainly of polysaccharides (Zech et al., 1997) while the humins (H) are generally composed of modified lignins (Almendros et al., 1996). This distribution varies a little as a function of soil management and environmental conditions (Zech et al., 1997). Since the C distribution in the different functional groups in humic acids is altered in a significant manner, this variation can be used for the evaluation of the process of organic matter humification (Zech et al., 1997). However, studies of organic matter using ^{13}C NMR spectroscopy have generally indicated only the chemical nature of organic C. Pyrolysis-gas chromatography / mass spectroscopy (Py-GC/MS), on the other hand, has successfully been applied to the characterization of organic matter (Saiz-Jimenez, 1994). Py-GC/MS involves chromatographic separation of pyrolysis products into single components and mass spectral data obtained for each component (Gottlieb et al., 1983). Thus, this technique allows the characterization of actual molecules which constitute the HA. However, the interpretation of pyrolysis data requires a detailed knowledge of the pyrolysis behaviour of the compounds under study because many products can be originated from thermal secondary reactions (Saiz-Jimenez, 1994). The combination of spectroscopic methods with thermolytic fractionation is a powerful tool in determining structural changes in humic substances and also in the identification of specific organic compounds (Kögel-Knaber, 2000; Davies et al., 2001).

The modifications in the natural soil organic matter due to the use of urban organic residue in cultivated lands have been investigated, with special attention to pollution compounds (e.g., incorporation of polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) (Felix-Henningsen et al., 1993; Bechmann & Grunewald, 1995) and dimethylphthalate (Leinweber et al., 1996). However, investigations of humified organic matter of tropical soils with sewage application employing ^{13}C-NMR and Py-GC/MS were not carried out. In previous works, humic acids extracted from sewage sludge and compost of municipal wastes from Rio de Janeiro were characterized mainly by chemical analyses, spectroscopic methods and scanning electron microscopy (Canellas et al., 2000a,b). These HA present high content of aromatic compounds and COOH groups and define the HA from urban sources as being the same type or similar to those of pedogenic origin. The Py-GC/MS analysis revealed the predominance of mono and diaryl esters in the aliphatic compounds. Aromatic products were originated from naphthalene and benzene derivatives. Furthermore, phthalate fragments were incorporated into the HA, probably due to organic matter contamination by plastics and the lipid fraction is constituted mainly of long chain fatty acids.

The purpose of this work is to evaluate the effect of anthropogenic organic matter (i.e., municipal compost and sewage sludge) addition on the structural features of the humic acids isolated from two different soils by elemental analysis, ^{13}C-NMR and pyrolysis – gas chromatography / mass spectrometry techniques.

MATERIAL AND METHODS

Soil samples: The soil samples used in this investigation weren take from the surface (0-20 cm) of the Ultisol (Ul) (Argissolo Vermelho Amarelo, according to the Brazilian Soil Taxonomy) at the Centro Nacional de Pesquisa em Agrobiologia - EMBRAPA-CNPAB, Seropédica (RJ), Brazil. This soil had a pH in H_2O of 5.5 and contained 16.0 g C, 1.0 g, N and 247 g clay kg^{-1}. The Oxisol (Ox) (Latossolo Vermelho Amarelo, according to the Brazilian Soil Taxonomy) soil sample was also collected from the surface (0-20 cm) in Piraí (RJ), Brazil. It had a pH in H_2O of 4.3 containing 24.4 g C, 2.3 g N and 537 g clay kg^{-1}. Previously, both soils were covered with pasture. Soil samples were amended with municipal compost and sewage sludge at the rate of 80 t ha^{-1} (dry basis). Details of the experimental conditions are described in Mazur (1997) and Oliveira (1998). The treatments were carried out with four replicates. After 24 weeks of incubation in 7 kg pots at room temperature and humidity at field capacity, the samples were dried. One set of soil samples was analyzed at the same experimental conditions but without organic residue additions. After approximately 80 weeks of storage time, the humic substances were extracted under N_2 atmosphere with a 0.5 mol L^{-1} NaOH solution with a 1:10 soil sample: solvent ratio. The isolation of the HA was performed by the addition of HCl 6 mol L^{-1} to pH 1. The redissolution and reprecipitation of HA was repeated three times. HA purification was performed using a diluted mixture of HF–HCl solution, centrifuged at 3.000 *x g* for 20 min and dialysing against deionized water. Finally, HA was freeze-dried.

Elemental analysis: The elemental composition (C, H, N, O) of humic acids was determined by dry combustion using a Perkin Elmer 2400 CHN elemental analyzer in the Laboratory of Environmental Science in the State University of Northern Rio de Janeiro (UENF) in triplicate samples with approximately 10 mg and moisture - free (after liophilization the samples were kept under vacuum and P_2O_5 atmosphere). The amount of C, H and N was obtained directly from analysis, whereas O was calculated by difference, i.e., O%= 100-C%-H%-N% (ash free basis) and the results (± standard mean error) are summarized in Table 1.

Ash measurement: This was carried out by weighing the residues, after the humic acids had been dried under vacuum at 60ºC for 16 h and reduced to ash at 800ºC for 6 h. The ash contents of humic acid samples were estimated by the following equation: ash %= $(W_{800}/W_{60}) \times 100$ where W_{60} is the weight of humic acids sample after heating at 60ºC for 16 h and W_{800} is the weight of the sample residue after heating at 800ºC for 16 h.

^{13}C NMR: Quantitatively reliable ^{13}C NMR solution spectra were run on an AC-200 Bruker instrument in the Department of Chemistry of the Federal Rural University of Rio de Janeiro (UFRRJ) (50,30 MHz to ^{13}C) using a 90º pulse, inverse-gated decoupling, and relaxation delays of 2 s, 0.2 s acquisition time and size of 16 K and 200 000 transients per sample. The samples were prepared with 150 mg in 2 mL of 0.5 mol L^{-1} NaOH (1:1 $H_2O:D_2O$) solution. All spectra were obtained at room temperature. Chemical shifts (δ_C) are reported as parts per million (ppm) from tetramethylsilane (TMS). Spectra were divided into the following chemical shift regions: δ_C 10-50 (alkyl C), δ_C 50-110 (mono and di oxigenated C and nitrogenated C), δ_C 110-165 (aromatic and olefinic C) and δ_C 165-185 (carboxyl, amide and ester C) (Wilson, 1987). The variation of integration data of signals due to the treatment of a well-resolved FID (Fourier transformation, phasing and baseline correction) is 5 % (Kögel-Knaber, 2000).

Pyrolysis/gas chromatography-mass spectrometry: This was performed on a Hewlett

Packard 5890 II gas chromatograph in the Department of Chemistry of the UFRRJ, with a fused silica column (30 m x 0.25 mm i.d.) coated with DB5MS. Samples of 1.0 mg were introduced as solids using an injector to the SGE pyrojector and were pyrolysed at 600°C. The temperature of the column was kept at 5°C for 2 min., then raised at a rate of 5°C per min. up to 220°C and kept there for 15 min. Helium was used as a carrier gas at a flow rate of 1 mL mim^{-1}. The injection mode was approximately 1:120 split ratio. Mass spectra (1scan/s) were recorded under electron impact at 70 eV condition. The assignment of compounds released from the pyrolysis was based on a search of a mass spectral library (Nist) and by comparison with other published collections of Pyrolysis-CG/MS data of similar samples (Saiz-Jimenez & Leeuw, 1986; 1987; Lu et al., 2000). The structural attribution for mass spectra fragmentation is not unequivocal but is consistent with the Pyrolysis-GC/MS data state of art (Davies et al., 2001).

RESULTS AND DISCUSSION

Elemental analysis: The elemental composition of humic acids and their atomic ratios are presented in Table 1. The data show that C contents of humic acids range from 487.5 to 579.5 g kg^{-1} and decreases in the order: Ox > Oxc > Oxs and Ul > Ulc > Uls. The H contents are in the range of 25.3 to 50.4 g kg^{-1} and decrease similarly to C content. Nitrogen contents range from 20.5 to 28.8 g kg^{-1} and increase in the order: Oxs < Oxv »Ox and Uls < Ulc » Ul. The O/C, H/C and C/N atomic ratios are often used to identify humic substances from different sources and to monitor structural changes of humic substances in different environments (Steelink, 1985). The O/C ratio is believed to be an indicator of the carbohydrate and carboxylic acid contents of humic acids. A decrease in O/C ratio suggests a decrease in the degree of oxidation of humic substances (Kononova, 1982). The values of this ratio increase when sewage sludge or compost are added to the Oxsoil. However, compost addition to the Ul soil does not alter the O/C ratio whereas sewage sludge does increase O/C ratio for this soil. According to the condensation theory of humification processes, humic substances gradually lose O functional groups and increase in aromaticity by condensation reactions. Addition of organic residue probably promotes changes in the humification pathway with an increase in oxidation reactions. According to Belzile et al. (1997), the value of the H/C ratio can also be used to assess the degree of maturity of humic substances. In Oxsoil samples, H/C ratios decrease in the order: Ox > Oxc > Oxs and in Ul soil samples in the order: Uls > Ul » Ulv. Dehydrogenation processes are more evident in the HAs isolated from soil samples treated with sewage sludge. C/N ratio is considered to be an indicator of the sources of humic substances in natural systems. The values of C/N for HA from Ul soil samples are higher than those for HA extracted from soil samples amended with organic residues. This suggests that other microbiological reactions occur during the incubation period. The C/N ratios for the Ox soil samples do not change with addition of compost but increase with sewage sludge. The elemental composition data can be interpreted only in a general way in terms of structural features of humic acids.

^{13}C NMR analysis: Integration of ^{13}C NMR signals is summarized in Table 2 (spectra not shown). The intensity of the signals corresponding to alkyl content in the HA is relatively high, mainly for the Ul soil samples. These signals can be attributed to the presence of long alkyl chains produced probably by non-degraded plant biopolymers, such as suberins and plant waxes (Wilson, 1987). HA extracted from municipal compost soil samples present significant reduction in this region. HA from sewage sludge

Table 1. Elemental composition, atomic ratios and ash content in humic acids extracted from ultisol (Ul) and oxisol (Ox) with municipal compost (Ulc and Oxc) and sewage sludge (Uls and Oxs) addition

	Ox	Oxc	Oxs	Ul	Ulc	Uls
C (%)	531 ± 3(1)	518 ± 5.8	509 ± 3.3	579 ± 1.0	558 ± 4.1	487 ± 3.3
H (%)	47 ± 0.7	40 ± 0.7	25 ± 0.5	50 ± 0.9	50± 0.3	46 ± 0.4
N (%)	23 ± 0.6	23 ± 0.7	20 ± 0.6	23± 0.4	29 ± 0.1	25 ± 0.8
O (%)	359 ± 11.1	385 ± 10.1	407 ± 13.0	347 ± 21.5	323 ± 13.1	399 ± 11.5
C/N	23.3	22.9	24.8	25.1	19.4	19.7
H/C	1.1	0.9	0.6	1.0	1.1	1.3
O/C	0.5	0.5	0.6	0.4	0.4	0.6
Ash (%)	4.0 ± 1.0	3.5 ± 0.8	3.8 ± 0.9	5.3 ±. 0.8	4.0 ± 0.2	4.2 ± 0.3

(1) Standard mean error.

soils show a smaller decrease in sp^3 C atoms of polymethylene chains. The spectral region corresponding to C bound to one or two O or N atoms, commonly attributed to carbohydrate and/or polyalcohols and amino acids in humic acids, revealed an increase in the integration areas when organic residues were amended. These results are consistent with the O/C ratios, being indicative of HA transformation by chemical and biological reactions during the incubation time (Kögel-Knaber, 1993). Hatcher & Spiker (1988) discussed the incorporation of polysaccharides in the humified fractions. Beyer et al. (1996) observed analogous phenomena in sewage sludge soils and attributed them to the incorporation of microbial products. The intensity of signals corresponding to aromatic carbons remain nearly constant in all soil samples. However, the intensity of ^{13}C signals attributed to carboxylic/carbonyl groups content decrease in the Ox soil samples with organic additions and remains nearly constant in the Ul soil samples. Thus, ^{13}C NMR spectra of humic acids provide a balance for the structural modifications during transformation in the course of the incubation time and show a clear reduction in the C alkyl content and a significant increase in O and di-O alkyl C atoms, suggesting the presence of more labile fractions in AH from soils samples treated with urban organic residues. Table 2 shows an increase in O and di-O alkyl C:C-alkyl ratio in HA´s isolated from soil samples treated with both organic residues and confirm the increase in O content observed by elemental analysis, exceptionally in Ulc HA.

Pyrolysis gas chromatography mass spectrometry: Due to the presence of a vast number of compounds, it is not possible to label all peaks in the chromatogram and, consequently, only the ones considered important were selected, as shown in Figure 1. The HA pyrograms can be characterized by seven groups of products, namely lignin derivatives (L), carbohydrate derivatives (C), nitrogen compounds (N), aromatic compounds (Ar), aliphatic (Al) and phthalate derivatives (Pht). The peak number in the pyrograms corresponds to the numbers mentioned in Table 3. The compounds corresponding to main peaks are suggested on the basis of comparative analysis with mass spectra recorded in the Nist library, only when the similarity was above 90 % and the fragmentation pathway was consistent with structural attribution. It is not unequivocal but it is consistent with state of the art of humic acids mass spectral analysis (Schulten, 1999; Davies et al., 2001).

Lignin derivatives such as guayacil, coniferil, creosol and tocoferol units are represented in the pyrograms. The more abundant products formed from carbohydrates are methyl furfural and furfuralaldeyde, along with other minor furan derivatives. These are typical products derived from carbohydrates (Bracewell & Robertson, 1976). According to Stuczynski et al. (1997). Changes in the amount of furan-related compounds are consistent with removal of reducing sugars by water extraction and also decrease in amino sugar content of soil organic matter. This suggests that furans can be used as biomarkers for monitoring environmental stress. Furan compounds arise from microbial degradation of polysaccharides from soil organic matter (Hayes et al., 1989). The most abundant phenol products are methyl phenol and ethyl methoxy phenols. Phenols comprise another diagnostic group of pyrolitic products and their low observed content suggests that they are derived mainly from hydrolysis of carbohydrates rather than lignin-derived subunits (Stuczynski et al., 1997). The main N identified products are heterocyclic compounds such as imidazol, pyrrole, tetrahydroquinoline, benzoxazol and pirydine derivatives. Dihydroxybenzene, propenyl benzene, methyl benzene, naphthalene and xylene are the major aromatic compounds in the HA. The major aliphatic products are alkanes and long-chain alkyl monoesters. These are typical products arising from the pyrolisis of polyethylene structures, such as lipids in humic substances. A decrease in the long alkyl chain region observed by ^{13}C NMR, with addition of urban organic matter to HA from

Table 2. Relative percentage peak areas in the NMR ^{13}C spectra from humic acids extracted from ultisol (Ul) and oxisol (Ox) with municipal compost (Ulc and Oxc) and sewage sludge (Uls and Oxs) addition

δC (ppm)[1]	Ox	Oxc	Oxs	Ul	Ulc	Uls
0-48	25.95	21.06	23.25	38.43	19.24	27.42
50-110	23.40	31.64	27.81	8.87	28.10	21.57
110-140	32.35	34.00	33.73	33.74	33.77	32.42
160-185	18.28	12.91	15.19	18.92	18.87	18.57
Ar[2]	39.6	39.2	39.8	41.6	41.6	39.8
O-alkyl/C-alkyl[3]	0.9	1.5	1.2	0.2	1.5	0.8

[1] Chemical shift regions according Wilson (1987): δ_C 10-50 (alkyl C), δ_C 50-110 (mono and di oxigenated C and nitrogenated C), δ_C 110-165 (aromatic and olefinic C) and δ_C 165-185 (carboxyl, amide and ester C). [2] Ar: aromaticity degree (δ_C 110-160/δ_C 0-160)*100. [3] (δ_C 50-110/δ_C 0-50).

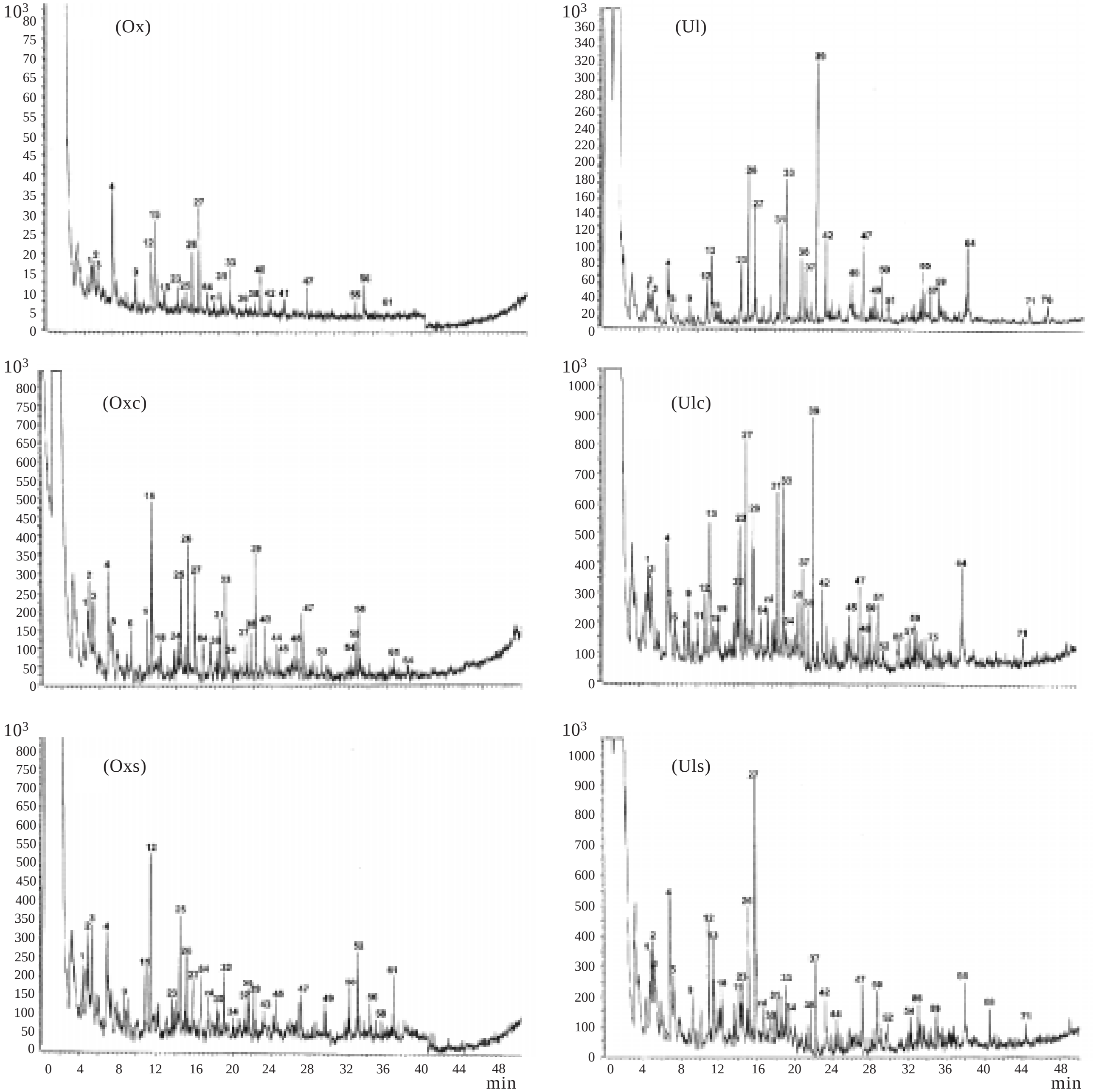

Figure 1. Py-GC/MS profile of humic acids isolated from Oxisol (Ox) Oxisol plus compost (Oxc) Oxisol plus sewage sludge (Oxs), Ultisol (Ul), Ultisol plus compost (Ulc), and Ultisol plus sewage sludge (Uls).

Ul, is consistent with the relative decrease in the intensity of the main peaks by Pyrolysis-CG/MS, but not in Ox soil samples. The content of aliphatic hydrocarbons in these amended soils is higher than in soils without addition of urban organic residues. According to this observation, the decrease observed in the δ_C 0-40 region by ^{13}C NMR signal integration reflects the balance of sp^3 carbon atoms in long alkyl chains. Pyrolysis-CG/MS data confirm that the new aliphatic structures are incorporated to HA structures from Oxs soil.

Figure 2 shows the relative abundance of the compounds represented in the pyrograms of the HA. Table 3 shows the major compounds and their main peaks in the mass spectra. Relative abundance of 1 is attributed to the intensity of the peaks in the pyrograms of the HA isolated from soil samples not treated with residues. Relevant differences are found for the relative distribution of compounds in the humic acids from soils treated with residues and non-treated soils. Thus, for the Ultisol treated with residues, a decrease in the amount of phenolic and

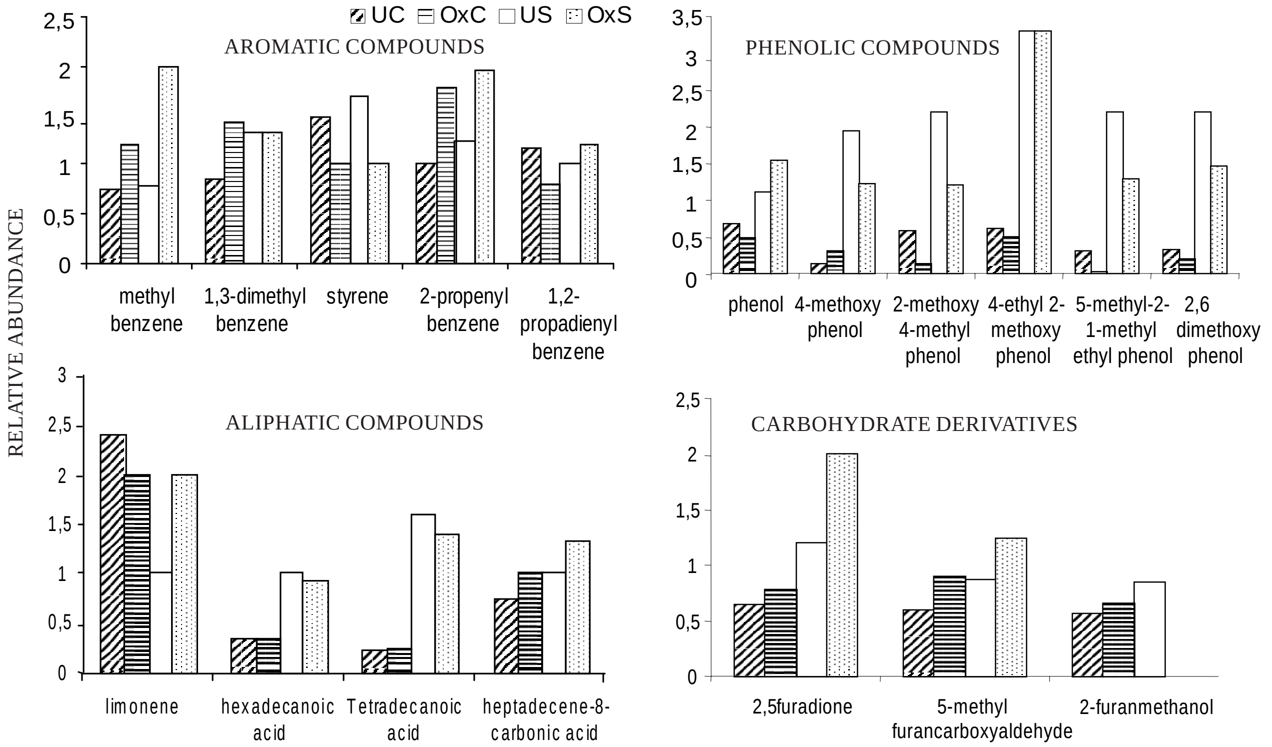

Figure 2. Relative abundance of aromatic compounds, furan derivatives, phenolic and aliphatic compounds in the pyrograms of humic acids from soil samples with and without organic residues. The relative abundance was considered 1 to peak in the chromatograms from HA pyrolitic products without organic residues addition (not shown in the graphics). Abreviattions: Humic acids extracted from: Ox: oxisol; OxC: oxissol with 80 t ha^{-1} municipal compost, OxS: oxissol with 80 t ha^{-1} sewage sludge. U: ultisol; UC: ultisol with 80 t ha^{-1} municipal compost, US: ultisol with 80 t ha^{-1}sewage sludge.

Table 3. Gas chromatography – mass spectrometry (GC/MS) data from the humic acids obtained by pyrolysis$^{(1)}$

N. peak	Retention time	Compounds	Elem.$^{(2)}$	m/z (intensity in %)
	min			
2	5.35	methyl benzene	C_7H_8	92(60), 91(100), 77(10), 15 (5)
4	7.3	2,5 furandione	$C_4H_4O_4$	98(10), 54(50), 26(100)
6	8.13	1,3-dimethyl benzene	C_8H_{10}	106(50), 91 (100), 77 (5), 51(20)
8	8.80	styrene	C_8H_8	104(100), 97 (1), 78(80), 77(30), 51(40)
12	10.98	5- methyl, 2- furancarboxaldehyde	$C_6H_6O_2$	110(80), 109(100), 51(80), 27(60)
13	11.41	phenol	C_6H_6O	94(100), 77(5) 66(50),39(50)
15	11.72	2-propenyl benzene	C_9H_{10}	118(85), 117(100), 78(60), 55(10)
16	13.32	limonene	$C_{10}H_{16}$	136(20), 121(20), 93(60), 68(100),
17	13.93	1,2-propadienyl benzene	C_9H_8	116(60), 115(80), 107(20), 42(100)
26	15.22	4-methoxy phenol	$C_7H_8O_2$	124(75), 109(100), 95(5), 81(70)
27	15.91	2- furanmethanol	$C_5H_6O_6$	98(70), 81(10), 68 (70), 53(60), 39(100)
31	18.56	2-methoxy-4-methylphenol	$C_8H_{10}O_2$	138(98), 123(100), 110(20) 95(40)
37	21.23	4-ethyl-2-methoxy phenol	$C_9H_{12}O_2$	152(45), 137(100), 77(20)
39	22.29	5-methyl-2-1methylethyl phenol	$C_{10}H_{14}O$	150(100), 135(95), 107(50)
40	22.75	1-2-hydroxy-5-methylphenyl ethanone	$C_9H_{10}O_2$	150(95). 135(100), 107(40), 77(50), 51(30)
42	23.27	2,6-dimethoxy phenol	$C_8H_{10}O_3$	154(100), 139(50), 111(30)
46	26.15	2-methoxy-4-2-propenyl phenol	$C_{10}H_{12}O_2$	164(100), 149(40), 137(20), 131(20)
49	28.30	2-propanone, 1-4-hydroxy-3-methoxyphenyl	$C_{10}H_{12}O_3$	180(30), 154(10), 137(100)
50	29.10	3-(3,4-dihydroxyphenyl)2-propenoic acid	$C_9H_8O_4$	180(100), 165(40), 137(30),
85	33.55	tetradecanoic acid	$C_{14}H_{28}O_2$	228(20), 185(30), 143(20), 129(50), 73(80), 60(80)
52	37.18	hexadecanoic acid	$C_{16}H_{32}O_2$	256(20), 213(20), 129(30), 60(60), 43(100)
54	41.71	heptadecene-8-carbonic acid	$C_{18}H_{34}O_2$	282(5), 26410), 125(10), 11(20), 83(60), 55(100)
55	42.02	octadecanoic acid	$C_{18}H_{36}O_2$	284(30), 255(10), 185(20), 129(40), 73(80), 43(100)

(1) Only those peaks with a similarity above 90% with the Nist library and consistent fragmentation pathway. (2) Elemental composition.

furan derivatives is observed. On the other hand, the opposite is observed for the Oxisol soil. This suggests that the higher content of clay in the Oxisol protects the original compounds from degradation. The relative abundance of aliphatic compounds corroborates this statement. The HA from the Ultisol has a higher amount of aliphatic compounds (Table 2). The relative abundance of aromatic compounds suggests an intense transformation of humic acids isolated from soils with addition of urban residues. The HA previously isolated from municipal compost and sewage sludge (Canellas et al., 2000a) show a high content of aliphatic C (äC 0-110) 63 and 56 %, respectively, and, consequently, has a lower degree of aromaticity (31 and 37 %, respectively) than the ones in the present study. Furthermore, the Py-GC/MS analysis of the HA from municipal compost and sewage sludge (Canellas et al., 2000 a) revealed the presence, in both cases, of phthalate derivatives in high amounts. These compounds were found in HA soil samples with organic residues addition but the peak intensity on the chromatograms was a very low revealing decomposition of phthalates (Inman et al., 1984) or dilution effect in soil. The chromatographic profile of the HA samples treated with urban residues after 24 weeks of incubation is similar to that one of the HA isolated from the organic residues, especially regarding aliphatic compounds. This observation corroborates the fact that addition of organic residues of anthropogenic origin induces changes in the structural features of humic acids.

CONCLUSIONS

1. The 13C NMR analysis allowed the clear observation of changes in the molecular composition of humic acids isolated from soil samples treated with both organic residues, mainly through the increase in the content of mono and dioxygenated C atoms and also the decrease in the CH_2 and CH_3 contents.

2. In addition, the Py-GC/MS technique allowed the observation of significant qualitative changes in the compounds originated from the thermal fragmentation of the HA. New compounds, such as lipids and furans, were detected with the help of this technique.

3. The combined use of 13C NMR spectroscopy and Py-GC/MS was effective in evaluating the changes in the molecular composition of HA which are brought about by addition of organic residues of urban origin.

LITERATURE CITED

ALMENDROS, G.; GUADALIX, M.E.; GONZÁLEZ-VILA, F.J. & MARTIN, F. Preservation of aliphatic macromolecules in soil humins. Org. Geochem., 24:651-659, 1996.

BAYER, C.; MIELNICZUK, J.; AMADO, T.J.C.; MARTIN-NETO, L. & FERNANDES, S.V. Organic matter storage in a sandy clay loam Acrisol affected by tillage and cropping systems in southern Brazil. Soil Tillage Res., 54:101-109, 2000.

BECHMANN, W. & GRUNEWALD, K. Organische schadstoffe in böden und substraten des rieselfeldgebietes südlich Berlins. Zeits. Pflanzen. Bodenk., 158:543-548, 1995.

BEUDERT, G.; KÖGEL-KABNER, I. & ZECH, W. Micromorphological, wet chemical and 13C NMR spectroscopic characterization of density fractionated forest soil. Sci. Total Environ., 81/82:401-408, 1989.

BELZILE, N.; JOLY, H.A. & LI, H. Characterisation of humic substances extracted from Canadian lake sediments. Can. J. Chem., 75:14-27, 1997.

BEYER, L.; CORDSEN, E.; BLUME, H.-P.; SCHLEUSS, U.; VOGT, B. & WU, Q. Soil organic matter composition in urbic anthrosols in the city of Kiel, NW-Germany, as revealed by wet chemistry and CPMAS ^{13}C-NMR spectroscopy of whole soil samples. Soil Technol., 9:121-132, 1996.

BRACEWELL, J.M. & ROBERTSON, G.W. A pyrolysis-gas chromatography method for discrimination of soil humus types. J. Soil Sci., 27:196-205, 1976.

CANELLAS, L.P.; SANTOS, G.A.; RUMJANEK, V.; MORAES, A.A. & OLIVARES, F.L. Avaliação de características de ácidos húmicos extraídos de resíduos de origem urbana I: métodos espectroscópicos (UV-VIS, IV, RMN ^{13}C-CP/MAS) e microscopia eletrônica de varredura. R. Bras. Ci. Solo, 24:741-750, 2000a.

CANELLAS, L.P.; SANTOS, G.A.; RUMJANEK, V. & MORAES, A.A. Avaliação de características de ácidos húmicos extraídos de resíduos de origem urbana II: pirólise acoplada à cromatografia gasosa-espectrometria de massas. R. Bras. Ci. Solo, 24:751-757, 2000b.

CERETTA, C.A.; BAYER, C.; DICK, D.P.; MARTIN-NETO, L. & COLNAGO, L.A. Métodos espectroscópicos. In: SANTOS, G. A. & CAMARGO, F.A.O., eds. Fundamentos da matéria orgânica do solo: ecossistemas tropicais & subtropicais. Porto Alegre, Gênesis, 1999. p.293-336.

DAVIES, A.N.; KUCKUK, R.; HILL, W.; NOLETE, J. & BURBA, P. A comparison of various pyrolysis experiments for the analysis of reference humic substances. J. Anal. Appll. Py., 60:145-157, 2001.

FELIX-HENNINGSEN, P.; WILBERS, A. & CRÖBMANN, G. Polyder Rieselfelder der Stadt Münster (Westfalen). Zeits. Pflanzen. Bodenk., 156:115-121, 1993.

HATCHER, P.G. & SPIKER, E.C. Selective degradation of plant molecules. In: FRIMMEL, F.H. & CHRISTMAN, R.F., eds. Humic substances and their role in the environment. Chichester, Willey, 1988. p.59-74.

HAYES, M.H.B.; MaCCARTHY, P.; MALCOM, R.L. & SWIFT, R.S. Humic substances II. In Search of structure. New York, John Wiley & Sons, 1989. 764p.

HEMPFLING, R. & SCHULTEN, H.-R. Chemical characterization of the organic matter in forest soil by curie point pyrolysis-CG/MS and pyrolysis field ionisation mass spectrometry. Org. Geochem.,15:131-145, 1990.

INMAN, J.C.; STEPHEN, D.; STRACHAN, D.; SOMMERS, L.E. & DARRELL, W.N. The decomposition of phthalate esters in soil. J. Environ. Sci. Health, B19:245-257, 1984.

GOTTLIEB, O.R.; BRAZ-FILHO, R.; ALENCAR, J.W. & CRAVEIRO, A.A. Introduction a la espectrometria de masa de sustancias orgánicas Washington, Organization of American States, 1983. 138p.

KÖGEL-KNABER, I. Biodegradation and humification processes in forest soil. In: BOLLAG, J.M. & STOTZKY, G., eds. Soil Biochemistry. New York, Marcel Dekker, 1993. v.8. p.101-135.

KÖGEL-KNABER, I. Analytical approaches for characterizing sol organic matter. Org. Geochem., 31:609-625, 2000.

KONONOVA, M.M. Matéria orgánica del suelo; su naturaleza, propiedades y métodos de investigación. Barcelona, Oikos – Tau, 1982. 365p.

LEINWEBER P.; BLUMENSTEIN, O. & SCHULTEN, -H.R. Organic matter composition in sewage sludge farm soils investigations by ^{13}C-NMR and pyrolysis-field ionisation mass spectrometry. Eur. J. Soil Sci., 47:71-80, 1996.

LU X., Q.; HANNA, J.V. & JOHNSON, W.D. Source indicators of humic substances: and elemental, solid state ^{13}C CP/MAS NMR and Py-CG/MS study. Appl. Geochem., 15:1019-1033, 2000.

MAZUR, N. Níquel, chumbo, zinco e cobre em solos que receberam composto de resíduos sólidos urbanos. Viçosa, Universidade Federal de Viçosa, 1997. 128p. (Tese de Doutorado)

OLIVEIRA, C. Avaliação do potencial de contaminação de dois solos agrícolas com lodo de esgoto enriquecido com cádmio, chumbo e zinco. Seropédica, Universidade Federal do Rio de Janeiro, 1998. 186p. (Tese de Doutorado)

PRESTON, C.M. Applications of NMR to soil organic matter analysis: history and prospects. Soil Sci., 161:144-165, 1996.

PRESTON, C.M.; HEMPLING, R.; SCHULTEN, H.R.; SCHNITZER, M.; TROFYMOW, J.A. & AXELSON, D.E. Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionisation mass spectrometry. Plant Soil, 158:69-82, 1994.

SAIZ-JIMENEZ, C. Application of pyrolysis-gas chromatography/mass spectrometry to soil chemistry. In: INTERNATIONAL CONGRESS OF SOIL SCIENCE, Acapulco 3, 1994. Procedings. Acapulco, 1994. p.65-84

SAIZ-JIMENEZ, C. & LEEUW, J.W. Chemical characterisation of soil organic matter fractions by analytical pyrolysis-gas chromatography – mass spectrometry. J. Anal. Appl. Pyrol., 9:99-119, 1986.

SAIZ-JIMENEZ, C. & LEEUW, J.W. Chemical structure of soil humic acids as revealed by analytical pyrolysis. J. Anal. Appl. Pyrol., 11:367-376, 1987.

SANTOS, G.A. & CAMARGO, F.A.O. Macromoléculas e substâncias húmicas. In: SANTOS, G.A. & CAMARGO, F.A.O., eds. Fundamentos da matéria orgânica do solo: ecossistemas tropicais & subtropicais. Porto Alegre, Gênesis, 1999. p.27-40.

SCHULTEN, H-R. Analytical pyrolysis and computational chemistry of aquatic humic substances and dissolved organic matter. J. Anal. Appl. Pyrol., 49:385-415, 1999.

STEELINK, C. Implications of elemental characteristics of humic substances. In: AIKEN, G.R.; McKNIGHT, D.M.; WERSHOW, R.L. & MAcCARTHY, P., eds. Humic substances in soil, sediment, and water. New York, Willy Sons, 1985. p.457-476.

STUCZYNSK, T.I.; McCARTY, G.W.; REEVES, J.B. & WRICH, J.B. Use of pyrolysis GC/MS for assessing changes in soil organic matter quality. Soil Sci., 162:97-105, 1997.

WILSON, M.A. NMR techniques and applications in geochemistry and soil chemistry. Oxford: Pergamon Press, 1987. 353p.

ZECH, W.; ZIEGLER, F.; KÖBEL-KNABER, I. & HAUMAIER, L. Humic substances distribution and transformation in forest soils. Sci. Total Environ., 117/118:155-174, 1992.

ZECH, W.; SENESI, N.; GUGGENBERGER, G.; KAISER, K.; LEHMANN, J.; MIANO, T.M.; MILTNER, A. & SCHROTH, G. Factors controlling humification and mineralization of soil organic matter in the tropics. Geoderma, 79:117-161, 1997.