Biogeochemistry **69:** 159–174, 2004.

Spectroscopic properties of sedimentary humic acids from a salt marsh (Ria de Aveiro, Portugal): comparison of sediments colonized by *Halimione portulacoides* (L.) Aellen and non-vegetated sediments

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Received 16 April 2003; accepted in revised form 12 August 2003

Key words: Halophytes, Humic substances, Salt marshes, Sediments, Spectroscopic analysis

Abstract. The influence of the colonization of salt marsh sediments with *Halimione portulacoides*, on the composition of the sedimentary humic acids was evaluated. For this purpose, cores of colonized and non-colonized sediments from a salt marsh in Ria de Aveiro (Portugal) were collected, and the humic acids of different layers were extracted and characterized by Fourier transform infrared, synchronous molecular fluorescence ($\Delta\lambda = 60$ nm) and UV-visible spectroscopies and also by elemental analysis. The infrared spectra suggest the presence of more peptide residues and carbohydrates in the sedimentary humic acids from surface and around the plant roots at the site colonized by *H. portulacoides*, when compared with the humic acids from the depth-equivalent sediment layers at the non-colonized site. The higher content of protein-type materials is confirmed by the lowest values of C/N ratios and the highest relative intensities of a band at $\lambda_{exc} = 280$ nm in the fluorescence spectra. The lowest ε_{280} values obtained in the UV-visible spectra, and the infrared spectra suggest a lower aromatic content of the humic acids from the colonized site.

Introduction

Salt marshes have a high net primary production with a large contribution from plant roots growth. These estuarine subsystems may play a role of effective filters and nutrient transformers, where the autotrophic organisms convert the dissolved inorganic nitrogen (NH_4 and NO_3), from land runoff, to their organic forms (Schlesinger 1991, p. 245). Considering that the major portion of the total energy flow in these environments is by the decompositional way (Packham and Willis 1997), much of this organic matter will be remineralized or transformed into other organic forms, by heterotrophic bacteria. Some of the organic compounds produced by living organisms will give rise to the formation of humic substances through complex pathways not yet completely clarified. These substances constitute the major fraction of the organic matter pool in aquatic environments (Kördel et al. 1997; Alberts and Takács 1999; Komada et al. 2002), and have been considered as

an important supplier and storehouse of energy and nutrients for plant roots and microorganisms (Aiken et al. 1985; Schnitzer 1985; Alberts and Filip 1994). The utilization of organic matter by the decomposers depends on its chemical composition, molecular size and inorganic nutrient concentrations (Amon and Benner 1996). Then, the molecular composition of sedimentary humic substances may be an important factor concerning the nutrient fate in salt marshes.

In estuarine salt marshes, the composition of sedimentary humic substances may reflect the contribution of complex mixtures of organic material coming from the rivers and the sea or produced 'in situ'. The sediment colonization with different species of plants may promote the introduction of different biochemical residues, which can be incorporated into the sedimentary humic matter, especially in the rizosphere zone. The composition and molecular characteristics of the humic matter will then reflect the contribution of the colonizing plants in the sediment, with implications to the availability of organic matter for microbial decomposition. The incorporation and progressive stabilization of polysaccharides and peptides of plant and microbial origin in soil humic fractions has been reported by Spaccini et al. (2000). However, there are only a few studies reported about the influence of salt marsh plants on the composition of sedimentary humic substances.

Filip et al. (1988) found a close similarity in composition and structural components between the humic substances isolated from the fresh and dead *Spartina alterniflora* and the humic acids from the colonized mud, despite the higher aliphatic content of the plant humic substances. The same authors verified that these humic substances were directly released to the seawater and modified by the activity of epiphytic microflora, increasing their aromatic content and loosing proteinaceous compounds and carbohydrates (Filip and Alberts 1988, 1994). Goñi and Thomas (2000) studied the organic matter along a transect of contrasting vegetation extending from upland forest soils to salt marsh sediments in an estuary at North Inlet, USA, and they verified the occurrence of important differences in the composition of the samples reflecting the predominant type of vegetation at each site.

All the above-mentioned studies showed that salt marsh plants might contribute to the humification process in the sediments. However, these studies are focused in United States salt marshes dominated by one single plant species, the cordgrass *S. alterniflora* and different species of salt marsh plants may be a source of organic material with different composition. Besides, as marsh plants are local sources of precursor compounds for humification reactions, the presence or absence of colonizing plants in sediments located nearby may induce small-scale variations in the composition of sedimentary humic substances, which have not been addressed in those studies. Those variations can arise not only due to the aboveground plant production but also due to the belowground production from root growth and decay. The rhizosphere will then be a source of fresh organic material to the surrounding sediments, and may induce differences in the composition of the humic substances, relatively to those from the depth-equivalent layer of a non-vegetated sediment located nearby. In consequence, the compositional trends of sedimentary humic substances along vertical profiles in those two sites may be different. As far as we know, this subject has not been investigated in the literature. This work aims at

evaluating small-scale variations in the composition of humic substances in salt marsh sediments, associated to the presence or absence of colonizing plants, and particularly those associated to the importance of the rhizosphere as a source of fresh humic precursors to the surrounding sediments.

Therefore, in this work we proceeded with the extraction and characterization of sedimentary humic acids from different depths of a vegetated and a non-vegetated sediment, including the depth corresponding to the highest abundance of plant root material. The humic acids characterization was performed by elemental analysis and synchronous molecular fluorescence, UV-visible and Fourier transform infrared spectroscopies.

The salt marsh studied is situated in Ria de Aveiro (Portugal). The vegetated sediment was colonized with *Halimione portulacoides*, which is an abundant species in this salt marsh and also around the world (Waisel 1972, p. 296). *Halimione* plants are low perennial shrubs of the *Chenopodiaceae*, occurring over a wide range of soil types on inundated sites as well as those with good drainage. This species is anemophilic and its flowering occurs during late summer and autumn, depending on temperature. Seed germination is best in fresh water, but their growth is stimulated by low NaCl concentrations (Waisel 1972, p. 296).

It is expected that this study will contribute to a better knowledge of the biogeochemical processes in salt marshes.

Methods

Sampling

Samples of sediment were collected from two sites (Figure 1) in a salt marsh situated in 'Largo do Laranjo' at the Aveiro Lagoon (Ria de Aveiro), Portugal. One site (site 1) was colonized by *H. portulacoides* and the other (site 2), located nearby, was a non-vegetated site. In each site, cores of sediment were collected and sliced into fractions of 5 cm. The fractions corresponding to the same depth of different cores were mixed and stored in plastic bags. The bags were tightly pressed around the sediment before being tightly closed with a knot.

At the laboratory, the plastic bags with the sediment were purged with nitrogen to remove any trace of air from inside and were stored in a freezer until freeze-drying. Humic substances were extracted from three sediment layers: layer A, the surface layer (0–5 cm); layer B, the layer corresponding to the higher content of plant roots (20–25 cm); and layer C, one deeper layer below the roots (50–55 cm).

Extraction and purification of humic substances

The extraction and purification of humic acids was performed according to the method proposed by the *International Humic Substances Society* (IHSS) for extraction of humic substances from soils (http://www.ihss.gatech.edu), with some

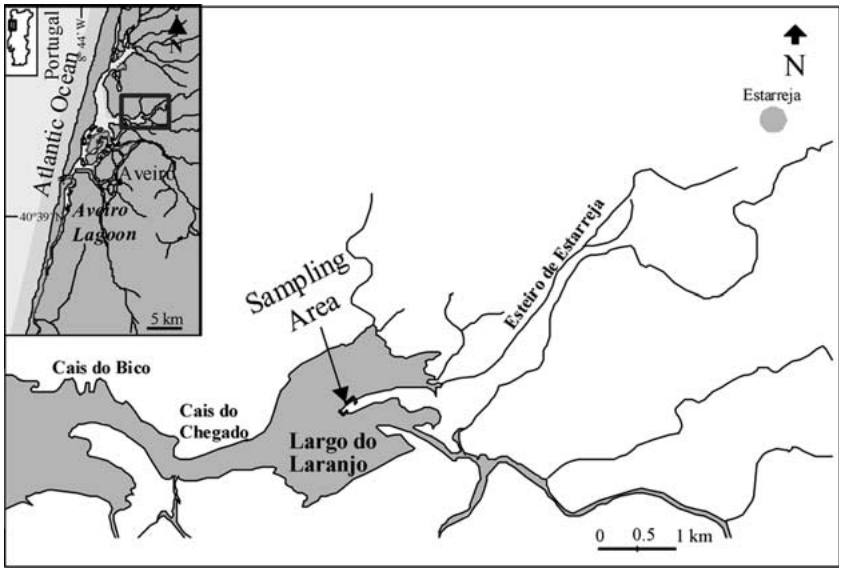

Figure 1. Localization of the sampling area at the saltmarshes of the Aveiro Lagoon (Ria de Aveiro).

modifications. The sediment was freeze-dried and its fine fraction (smaller than 1 mm), without roots, was separated from the rest of sediment for the extraction of humic substances.

A 1 mol l^{-1} HCl solution was added to 100 g of the fine fraction of dried sediment, until a pH 1–2 was reached; a volume of 10 ml/g of sediment was then completed with 0.1 mol l^{-1} HCl. This suspension was shaken for 1 h in a roller and the residue was allowed to settle. The supernatant with weakly bound calcium and metallic ions (http://www.ar.wroc.pl/~weber/ekstrak2.htm) and some acid soluble organic matter was then decanted. The non-decanted residue with humic material was then purged with nitrogen to prevent the oxidation of the humic material during the subsequent alkali extraction. For the alkali extraction, 1 mol l^{-1} NaOH was added to neutralize (pH 6–7) the sediment residue and a volume of 10 ml NaOH per gram of residue was completed with 0.1 mol l^{-1} NaOH, under an atmosphere of nitrogen. The suspension was then intermittently shaken for a minimum of 4 h and the alkali suspension was allowed to settle overnight.

The supernatant was collected by decantation, under nitrogen atmosphere, and neutralized with 6 mol l^{-1} HCl. At the end, the pH of the decanted extract was adjusted until a value between 1.0 and 1.3, to precipitate the humic acids, and the suspension was allowed to stand overnight.

The resultant suspension was centrifuged (10,000 rpm), for 30 min, in Teflon tubes with a capacity of 50 ml each. After the centrifugation, the supernatant was decanted and the precipitate with the humic acids was re-dissolved in a 0.1 mol l^{-1} NaOH solution, containing 0.2 mol l^{-1} KCl, inside a glove box under nitrogen

atmosphere. The KCl was added to NaOH to increase the ionic strength, provoking the flocculation of the colloidal inorganic material (non-humic material).

The suspended solids were removed by centrifugation (10,000 rpm, during 30 min) and the humic acids solution was decanted, inside a glove box under nitrogen atmosphere. The process was repeated until practically all the humic acids were separated from the inorganic matrix. The final solution of humic acids was acidified with 6 mol l^{-1} HCl to pH 1.0. The acidified solution was allowed to stand overnight and the supernatant was separated from the humic acids precipitate.

The humic acids were re-suspended in a small volume of 0.01 mol l^{-1} HCl and dialysed against distilled water until no significant increase of the water conductivity was observed. Finally, the purified and protonated humic acids were freeze-dried and stored until the characterization.

Chemical and spectroscopic characterization

Elemental analyses of the isolated humic acids and of the fine fraction (<1 mm) of the freeze-dried sediments were performed using a LECO CHNS-932 analyser. For the characterization by UV-visible and fluorescence spectroscopies, several solutions of humic acids in phosphate buffer (0.05 mol l^{-1}; pH 7), at different concentrations (5–20 mg l^{-1}), were prepared. The absorption spectra (700–200 nm) were obtained with an UV-visible spectrophotometer Shimadzu, UV 2101 PC model, using 1 cm pathlength quartz cells.

The synchronous molecular fluorescence spectra ($\Delta\lambda = 60$ nm, $\lambda_{exc} = 230$–500 nm) were obtained with a spectrophotometer JASCO (Tokyo, Japan), FP-770 model with a xenon lamp source. A 1 cm-cell was used as sample container. The instrument makes an automatic correction for the excitation lamp spectral profile and any temporal intensity variation, by using reference lightpath and dynode-feedback electronics. A fraction of excitation beam is directed to the reference detector associated with an internal rhodamine dye quantum counter to obtain wavelength independent reference signal, and any change at the excitation beam is used in real time to correct sample readings. The spectra were not corrected for the spectral response of the detection system. However, all the spectra were obtained in the same instrument at the same conditions, allowing comparisons between them.

These spectra were recorded at a scan speed of 200 nm/min, using excitation and emission slit bandwidths of 10 nm and 1 cm cuvettes. The fluorescence spectrum of the phosphate buffer (0.05 mol l^{-1}; pH 7) was subtracted from the spectra of all the humic acids solutions. To minimize spectral differences due to inner filter effects, spectra obtained at the same concentration were used for comparison between samples (9 mg/l). This concentration was found to be within linearity when fluorescence at wavelength maxima ($\lambda_{exc} = 454$ nm) is plotted against concentration (between 5 and 20 mg of humic acids/l). In addition, since the different samples of humic acids had different carbon percentages and different absortivities, a correction factor was used to minimize the effects of differences in optical densities. This correction factor takes into account the absorbance of the samples and can be

expressed as $10^{0.5(A_{excitation}+A_{emission})}$, where A is the absorbance at the excitation and emission wavelengths, respectively (Kalbitz and Geyer 2001). This expression is based on the assumption that the emission spectrometer views only a small illuminated volume in the centre of the 1 cm cell and so the effective pathlength of the excitation light is 0.5 cm. Similarly, the pathlength of the fluorescence light going through the sample to the emission monochromator is also 0.5 cm (McKnight et al. 2001). As the absorbances were measured using a 1 cm sample cell, they must then be multiplied by the factor 0.5.

The infrared spectra of samples were obtained on a Magna 550 Fourier Transform spectrometer using the KBr pellet technique (0.4 mg of the sample to 40 mg KBr). Spectra were performed at 4 cm^{-1} resolution and 100 scans were averaged to minimize noise. The sample compartment was purged with nitrogen for 15 min before recording spectra.

Results and discussion

Elemental composition

Table 1 shows the elemental composition (carbon and nitrogen contents) of the fine fraction (<1 mm) of the freeze-dried sediment samples. The highest values of carbon and nitrogen are observed in layers A and B of the colonized site, suggesting an introduction, into those sediment layers, of organic material resulting from above- and below-ground plant production.

The C/N ratios of humic acids ranged from 7.6 to 16.5 (Figure 2(a)). These values are similar to the data given by Filip et al. (1988) for sedimentary humic acids and plant decay humic substances (7.9–16.2). The C/N ratios of the humic acids from the layers A and B of the colonized site (7.6 and 11.1, respectively) are lower than the C/N ratios of the humic acids of the depth-equivalent layers from the non-vegetated sediment (10.5 and 15.3, respectively). In fact, the C/N ratio of the humic acids from the surface layer of the colonized site is similar to that obtained by Filip et al. (1988) for humic acids extracted from fresh tissues of *S. alterniflora* collected in marshes of Sapelo Island, Georgia (C/N = 7.9). These lower C/N ratios suggest the inclusion of protein-derived moieties in the humic acids from these layers of the colonized site and a lower degree of humification of these samples (Claus et al. 1999).

An increase of the C/N ratio with increasing depth was observed for both sites. Steelink (1985) referred this same trend as normal for humic acids from lake sediments and such a trend is indicative of an increase of the humification degree with depth (Kördel et al. 1997; Claus et al. 1999). Despite of an increasing trend of the C/N ratio with the depth at both sites, this trend occurs in a different manner according to their plant colonization. In the non-colonized site the trend between layers is: $A < B \approx C$, while at the colonized site the trend is $A < B < C$. These different trends are probably due to the influence of plant roots, at layer B of the colonized site, which may promote the inclusion of recent nitrogen-containing organic material.

Table 1. Carbon and nitrogen contends of the fine fraction (<1 mm) of sediment samples. Mean value and standard deviation (sd) of three replicates.

Sediment sample	C (%)	N (%)
1A	6.5 (sd = 0.4)	0.9 (sd = 0.4)
1B	6.6 (sd = 0.4)	0.6 (sd = 0.5)
1C	2.4 (sd = 0.4)	0.2 (sd = 0.4)
2A	2.2 (sd = 0.2)	0.2 (sd = 0.4)
2B	1.4 (sd = 0.5)	0.1 (sd = 0.4)
2C	1.7 (sd = 0.1)	0.1 (sd = 0.3)

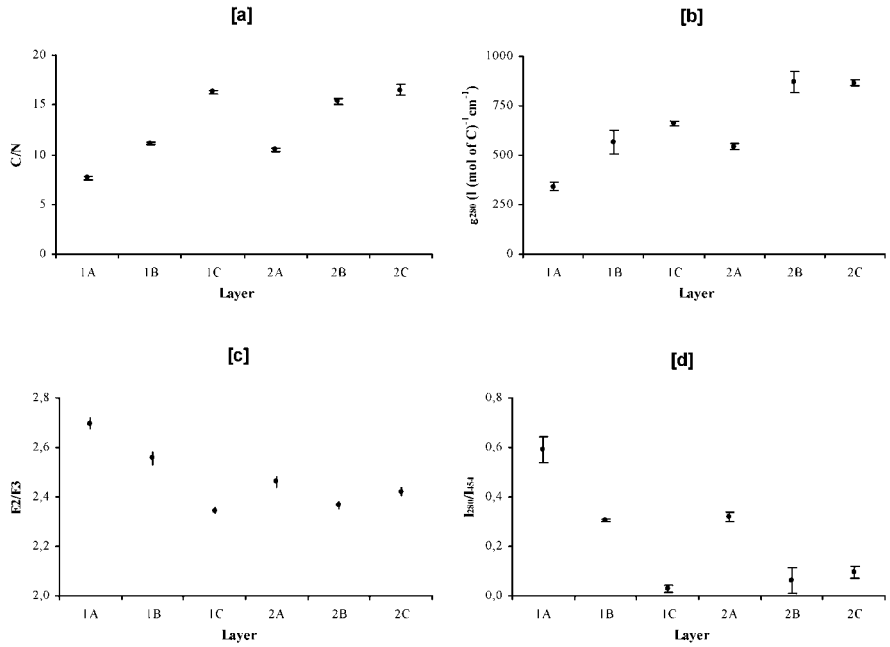

Figure 2. Compositional and spectroscopic characteristics of humic acids from the different sediment layers (A, B, C) of sites 1 (vegetated) and 2 (non-vegetated). 2(a). Mean values and uncertainties (95% confidence) of C/N ratio; 2(b). mean values and uncertainties (95% confidence) of ε_{280}; 2(c). Mean values and uncertainties (95% confidence) of E_2/E_3; 2(d). Mean values and uncertainties (95% confidence) of I_{280}/I_{454}.

UV-visible spectroscopy

The UV-visible spectra of the humic acids isolated in this work were featureless, with the absorbance decreasing with increasing wavelength. This behaviour is typical of the humic substances (MacCarthy and Rice 1985).

The specific absortivity at 280 nm (ε_{280}) has been used as an index of the degree of aromaticity (Chin et al. 1994). This wavelength was chosen because a large number of aromatic compounds (phenolic arenes, benzoic acids, aniline derivatives, polyenes and polycyclic aromatic hydrocarbons with two or more rings) absorbs in this UV region (270–280 nm) due to $\pi - \pi^*$ electronic transitions (Traina et al. 1990; Chin et al 1994; Peuravuori and Pihlaja 1997). Smaller values of ε_{280} may be indicative of higher carbohydrate content, since those compounds have a low absorbance at this wavelength (Peuravuori and Pihlaja 1997).

Figure 2(b) shows the ε_{280} values for the humic acids from the different sediment layers (A, B and C) of both sites. The humic acids from the surface sediment (layers 1A and 2A) and near the plant roots (layer 1B) have the lowest values of ε_{280}. These low values are probably caused by an intrusion of recent material, rich in carbohydrate or protein-derived moieties and poor in aromatic content, to the surface sediments from both sites and to the sediment around the plant roots at the colonized site.

McKnight et al. (2001) referred that microbially derived humic substances (humic substances resulting from extracellular release and leachate of algae and bacteria) have a lower aromaticity than terrestrially derived humic matter (resulting from decomposition and leaching of plants and soil organic matter). Besides, the polysaccharides are a class of microbially derived biomolecules that may be precursors of humic substances (McKnight et al. 1991). Claus et al. (1999) have also obtained microbially formed humic substances that resemble natural aquatic humic matter, but with more aliphatic constituents (carbohydrates and peptides) and less aromatic structures. Thus, the new organic material incorporated into the sediment surrounding plant roots may be provided by those roots (through their decomposition, leachates or exudates) and/or by the microbial communities, which may grow around them and which can transform the organic matter released.

The comparison between the vegetated and non-vegetated sites highlights that the humic acids from the vegetated site have the lowest values of ε_{280}, indicating a lower aromaticity (Chin et al. 1994; McKnight et al. 2001). This can be due to a lower degree of humification of these humic acids or to a higher degree of mixing of terrestrial humified materials, coming from the river and land runoff, with 'fresh' materials from salt marsh plants and their associated communities.

The ratio of the absorbance at 250 and 365 nm (E_2/E_3) has been used in some limnologic studies as an indicator for humification (De Haan 1983; Peuravuori and Pihlaja 1997) showing that this ratio decreased with the increase of the humification degree. In the present study, the humic substances with lower values of E_2/E_3 were those from the deepest layer of the vegetated site (1C) and from the intermediate and deepest layers of the non-vegetated site (2B and 2C), indicating a higher degree of humification in these layers (Figure 2(c)). Even the humic acids from layer 2A had a lower value of E_2/E_3 than those from 1B; this may be due to an effect of dilution of humified material with new fresh material coming from the decomposition of plant roots, in layer 1B.

The layers with the lowest values of E_2/E_3 are also those with the highest values of ε_{280}. Peuravuori and Pihlaja (1997) found a moderate linear correlation between

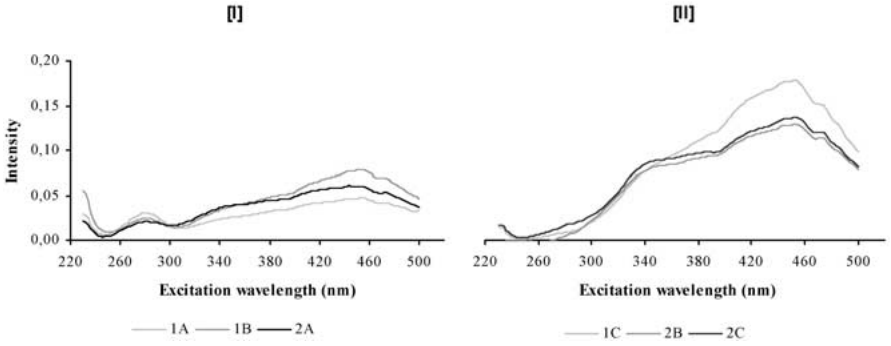

Figure 3. Synchronous spectra ($\Delta\lambda = 60$ nm) for the humic acids (9 mg/l) from different layers of sediment. There are two types of spectra: (I) spectra with a small band at $\lambda_{exc} = 280$ nm (layers 1A, 1B and 2A); and (II) spectra without a band at $\lambda_{exc} = 280$ nm (layers 1C, 2B and 2C).

the quotient E_2/E_3 and ε_{280} and between E_2/E_3 and aromaticity of humic substances, with the ε_{280} and aromaticity decreasing with increasing E_2/E_3 values. In the present study, the linear correlation between E_2/E_3 and ε_{280} represented by the equation $\varepsilon_{280} = 3689 - 1232E_2/E_3$ was shown to be quite acceptable ($r = 0.813$; $p = 0.049$). Such as in the work of Peuravuori and Pihlaja (1997) the correlation is negative, that is, an increase of E_2/E_3 implies a decrease in ε_{280}.

Fluorescence spectra

The synchronous molecular fluorescence spectra ($\Delta\lambda = 60$ nm) of the humic acids are shown in Figure 3. The spectra were divided into two types: those which exhibit a small band at $\lambda_{exc} = 280$ nm and those where that same band is absent. Fluorescence peaks within $\lambda_{exc} = 265$–285 nm/$\lambda_{em} = 300$–370 nm in three-dimensional excitation-emission-matrix (EEM) fluorescence spectra of natural waters have been attributed to proteinaceous materials (Coble et al. 1990; Mopper and Schultz 1993; Komada et al. 2002). Lu et al. (2003) obtained an intense peak at 285 nm in the synchronous fluorescence spectra ($\Delta\lambda = 30$ nm) of dissolved organic matter leached from periphyton, mangroves leaves and sawgrass from Southern Everglades wetlands. According to several authors (Coble et al. 1998; Baker and Genty 1999; Mayer et al. 1999; Reynolds 2003) the amino-acids tyrosine and tryptophan are the fluorophores accountable for most fluorescence signals in this region.

Mayer et al. (1999) obtained EEM fluorescence spectra from natural seawater samples and from artificial seawater solutions with monomeric amino-acids tyrosine and tryptophan and with animal and algal proteins. They recorded peaks at $\lambda_{exc} = 275$–280 nm with emission wavelengths at 305 and 340 nm on EEM spectra of all type of samples. These peaks were attributed to tyrosine and tryptophan, respectively. Mathews et al. (1996) did also observe a peak at $\lambda_{exc} = 280$ nm,

$\lambda_{em} = 320–350$ nm in the EEM spectra of the organic matter isolated from corals, which they attributed to tryptophan in proteins. The tryptophan residues have a higher quantum yield relative to tyrosine residues and, contrarily to tyrosine-type, the tryptophan-type fluorescence can appear when this fluorophore is combined with humic matter (Mayer et al. 1999). It is worth to notice that the difference between λ_{exc} and λ_{em} of the tryptophan peak in the EEM spectra reported is 40–70 nm. Besides, Reynolds (2003) obtained a good correlation between the fluorescence intensities at $\lambda_{exc} = 280$ nm in synchronous ($\Delta\lambda = 60$ nm) molecular fluorescence spectra of water samples and the levels of tryptophan in those waters, determined by HPLC analysis. This was the reason for the choice of a $\Delta\lambda = 60$ nm for recording the synchronous spectra of the sedimentary humic acids.

According to Ferrari and Mingazzini (1995), phenols, namely those resulting from lignin degradation, can also contribute to a peak at this λ_{exc} in the synchronous molecular fluorescence spectra of natural organic matter. However, the spectra which exhibit the band at $\lambda_{exc} = 280$ nm are those of the humic acids from the surface sediment layers of both sites and of the humic acids from the sediment around the roots, at the vegetated site. Those are the humic acids with lower C/N ratios and that supports the attribution of that peak at $\lambda_{exc} = 280$ nm to protein-derived structures in our samples. As also shown in Figure 3, all the spectra exhibit fluorescence maxima at $\lambda_{exc} = 453–455$ nm ($\lambda_{em} = 513–515$ nm), which is considered characteristic of humic substances. Maxima at 450–480 nm were also observed in the synchronous scan excitation spectra ($\Delta\lambda = 18–20$ nm) of soil humic acids (Miano et al. 1988; Chen et al. 2003) and aquatic humic acids (Senesi et al. 1989).

Figure 2(d) shows the values of the ratio between intensity of fluorescence at $\lambda_{exc} = 280$ nm and intensity of fluorescence at $\lambda_{exc} = 454$ nm (I_{280}/I_{454} ratio) for the humic acids from different layers of sediment. This ratio gives an indication of the fraction of proteinaceous material in relation to the more refractory material of each humic acid sample (Lu et al. 2003).

As it can be seen in Figure 2(d), the I_{280}/I_{454} values show a similar pattern to that obtained for E_2/E_3 ratio on UV-visible spectra and shown in Figure 2(c), with surface layers of both sites (1A and 2A) and the layer with more density of plant roots (1B) producing the highest values. Such as the E_2/E_3 values, these results suggest the presence of recent organic material in the humic fraction of these sediment layers. In fact, there is an inverse linear relationship between the ε_{280} values and the I_{280}/I_{454} ratio ($r = 0.893$; $p = 0.016$). This inverse linear trend may indicate that species of the deepest layers of both sites (1C and 2C) and the intermediate layer of non-vegetated site (2B) which absorb at 280 nm are not fluorescent at $\lambda_{em} = 340$ nm. Several authors (Ferrari and Mingazzini 1995; Coble et al. 1998; Komada et al. 2002; Chen et al. 2003) suggested that a progressive red-shift of the excitation/emission maxima of its fluorescent components accompanies increased age and humification of organic matter. This shift to longer wavelengths is due to the policondensation of the aromatic rings or/and the addition of certain functional groups to these structures, resulting in an increase of the delocalization of the π-electron systems (Ferrari and Mingazzini 1995; Komada et al. 2002).

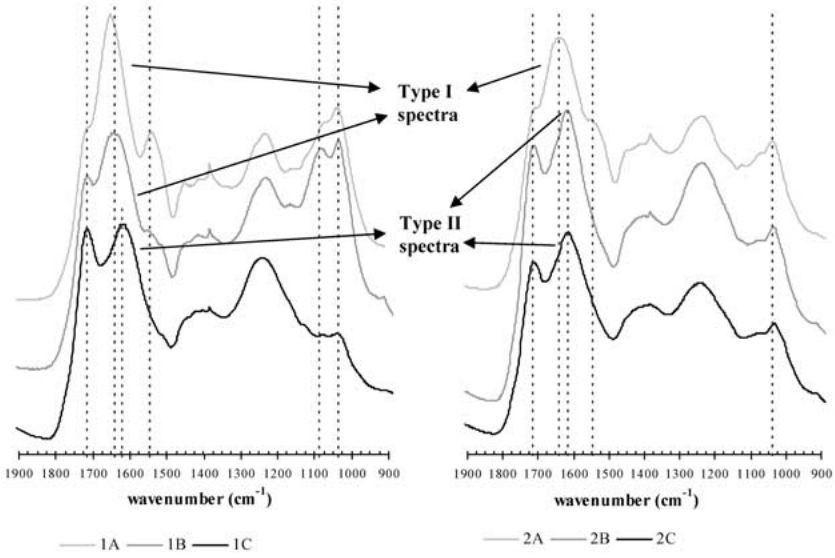

Figure 4. FTIR spectra in the zone 1900–400 cm^{-1} for humic acids from different layers of vegetated (site 1) and non-vegetated (site 2) sediment. Two types of spectra are identified: Type I – those with the amide bands (layers 1A, 1B and 2A); Type II – those without these bands and a shift to lower wavelength for the band at 1600–1700 cm^{-1} (layers 1C, 2B and 2C).

FTIR spectra

The infrared spectra obtained for humic acids studied are similar to spectra obtained in other studies with humic substances from several environments (Stevenson and Goh 1971; Peshel and Wildt 1988; Santos and Duarte 1998; Alberts and Filip 1994; Claus et al. 1999; Kalbitz et al. 1999). A broad and strong band at 3400 cm^{-1} is evident in all spectra. This band is typical of the spectra of natural humic substances and has been attributed to OH stretching of carboxyl, hydroxyl and phenol groups (Stevenson and Goh 1971; MacCarthy and Rice 1985; Peshel and Wildt 1988; Santos and Duarte 1998). As mentioned by Stevenson and Goh (1971) the existence of NH groups may contribute to this absorption band. Superimposed to this band there are a few signals in the 2970–2840 cm^{-1} region which have been attributed to asymmetric and symmetric CH stretching of methyl and methylene groups of aliphatic chains (Stevenson and Goh 1971; MacCarthy and Rice 1985; Santos and Duarte 1998).

As shown in Figure 4, the region between 1900 and 900 cm^{-1}, is where differences between samples become more apparent. In order to expedite the following discussion, the spectra in this region are classified into two types: type I, includes spectra of samples from layers 1A, 1B and 2A; and type II, includes spectra of samples from the other layers.

The first band in this region, at 1720 cm^{-1}, is mainly due the C = O stretching of carboxyl groups (Stevenson and Goh 1971; Plechanov et al. 1983; MacCarthy and Rice 1985; Peshel and Wildt 1988; Santos and Duarte 1998). The type I spectra have the lower relative intensities of this band.

The band centred at 1550 cm^{-1} appears only in the spectra of type I, being more intense in the spectrum of the humic acids from the surface layer of the colonized site. A band at this wavenumber has been attributed to amide groups and may indicate the presence of polypeptides-derived structures (Stevenson and Goh 1971; Peshel and Wildt 1988). Bellamy (1975) designated this band as the 'amide II band' on secondary amides and, according to this author, it can be assigned to an NH deformation mode and/or a C-N stretching mode. In addition to this band, amides show also an intense band at 1640 cm^{-1} (Stevenson and Goh 1971; Calace et al. 1999), due to the carbonyl absorption and it has been designated as the amide I band (Bellamy 1975). It is noticeable that the type I spectra where the amide I band is present, also show the amide II band. The other spectra also have a band in the 1600–1670 cm^{-1} zone but this band suffers a shift to shorter wavenumbers, approximately 1630 cm^{-1}. Absorption close to this wavenumber has been attributed to aromatic structures (Kalbitz et al. 1999). These results suggest the presence of more peptide structures in the samples from surface and from the layer around the roots and a higher aromaticity in the other samples. The evidence of the supply of the peptide units to the composition of sedimentary humic acids from surface layers and rizosphere is in agreement with the C/N ratio and fluorescence results. The ε_{280} results are in agreement with the higher aromaticity of the samples represented by type II spectra.

A comparison of type II spectra from the two sites shows that the ratio between the absorption bands at 1720 and 1630 cm^{-1} is higher in the sample 1C from the colonized site than in the samples 2B and 2C from the non-vegetated site. This suggests a higher content of carboxyl groups and a lower aromaticity in the sample 1C. This sample also exhibits a lower ε_{280} relatively to the other two samples (2B and 2C). Kalbitz et al. (2000) observed a linear relationship between ε_{280} and the absorbance at 1620 cm^{-1} and both parameters have been related to aromatic content.

The absorption bands at 1100–1090 cm^{-1} and 1050–1040 cm^{-1} have been attributed to C-O stretching of carbohydrate structures (Stevenson and Goh 1971; Peshel and Wildt 1988; Alberts and Filip 1994; Kalbitz et al. 1999). The higher relative intensities of these bands in the spectra of humic acids from the upper layers of the colonized site sediments (layers 1A and 1B), suggest that the presence of plants promotes the incorporation of these carbohydrate structures in the sedimentary humic substances.

Conclusions

Significant differences were observed between the humic acids of sediments from colonized and non-colonized sites of a salt marsh. At the colonized site the humic

acids from the surface sediment layer and from the sediment layer with higher density of plant roots revealed an incorporation of proteinaceous material in their composition. This incorporation is suggested by the lowest C/N ratios, the presence of a band at $\lambda_{exc} = 280$ nm (characteristic of aminoacids) in the synchronous fluorescence spectra and bands at 1550 and 1640 cm^{-1} (amide bands) in the FTIR spectra. These samples also showed a carbohydrate presence in their composition, as revealed by the lowest values of ε_{280} (lower aromaticity) and by the highest band intensities at 1050–1040 and 1090–1080 cm^{-1} (CO stretching of carbohydrates) in the FTIR spectra.

At the non-vegetated site only the humic acids from the surface layer exhibited the presence of peptide residues, but with less evidence than the sample of the same sediment layer of the colonized site.

At the deepest layers of the non-colonized (2B and 2C) and colonized site (1C) the sedimentary humic acids revealed to have a highest aromatic content and showed a higher degree of humification. These aspects were highlighted by the highest C/N ratio and ε_{280} values and by the highest fluorescence intensities when they are excited at 454 nm. The shift of the FTIR band at 1640 cm^{-1} to lower wavenumbers, together with the absence of the band at 1550 cm^{-1} also suggest the presence of aromatic structures.

Differences along depth are much more noticeable on the humic acids from colonized site than from non-vegetated site. At the non-vegetated site, the humic acids of the intermediate and deepest layer are quite similar, while at the colonized site, the humic acids from the intermediate layer do exhibit structural characteristics, which suggest the presence of peptide and carbohydrate moieties. This emphasizes the importance of the plant roots as a source of biogenic organic material, which is incorporated in the humic fraction of the sedimentary organic matter. These bioorganic materials may be released from the plant roots or provided by their decomposition due to the activity of local microbial communities.

In conclusion, it was shown that the presence of colonizing halophyte plants such as *H. portulacoides* in salt marsh sediments, leads to modifications in the humic acids composition of these sediments, relatively to non-colonized sediments located nearby. These modifications are quite visible in humic acids from the sediment surrounding plant roots. The colonizing plants promote an incorporation of new precursor material (carbohydrate- and protein-derived), which significantly modifies the composition and spectroscopic characteristics of the sedimentary humic acids. These modifications may affect the nutrients cycle. If the sediment conditions are favourable and the new material in humic acids is available to the microbial uptake, they will be used as a supplier of energy and nutrients for the decomposition process and the mineralization pathway will take place. The inorganic compounds produced can be consumed by the primary producers or exported to the open waters of the estuary. On the contrary, if the humic properties and the sediment conditions are not favourable for their microbial utilization, these ‘fresh’ materials can be incorporated into the humic pool for a long period and the salt-marsh sediment can behave as a sink of nitrogen. The dynamics of incorporation of fresh organic residues into the humic fraction of organic matter is not yet clarified

in terms of chemical structures and mechanisms involved. According to some authors peptide and polysaccharide residues can be stabilized in the humic fractions and protected from degradation, exhibiting long residence times in soils (Spaccini et al. 2000; Gleixner et al. 2002).

Acknowledgments

The authors wish to acknowledge the collaboration of the students Dóris Gomes, Martinha Santos, Paulo Domingos, Sofia Reis, Vanda Vaz Serra supported by the project PRAXIS/P/CTE/11025/98 from Fundação para a Ciência e Tecnologia of Ministério da Ciência e Tecnologia (FCT/MCT). One of the authors (A. Mendonça) wishes also to acknowledge a PhD grant from Centro de Zonas Costeiras e do Mar (CZCM).

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