Journal of Coastal Research	SI 39	1062 - 1065	ICS 2004 (Proceedings)	Brazil	ISSN 0749-0208

Acidity Data on Humic Substances from Distinct Environments: Methodology Considerations

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ABSTRACT

FERNANDES, A. N.; GIOVANELA, M.; SORIANO-SIERRA, E.J. and SIERRA, M.M.D., 2006. Acidity data on humic substances from distinct environments: methodology considerations Journal of Coastal Research, SI 39 (Proceedings of the 8th International Coastal Symposium), 1062 1065. Itajaí, SC, Brazil, ISSN 0749-0208.

Carboxylic (CA), phenolic (PhA) and total (TA) acidity contents of a set of marine (coastal zone), estuarine (mangrove), terrestrial (peat) and freshwater (lake) sedimentary fulvic (FA) and humic (HA) acids were determined via the Ca-acetate and the Ba-hydroxide indirect-titration methods. Total acidity contents were high, ranging from 10.50 to 26.00 meq g^{-1} and did not present the predicted trends being in disagreement with the elemental and infrared analysis results for this set of samples. Contrary to that expected, terrestrial samples exhibited higher carboxyl groups content than did aquatic samples and marine and lake samples presented higher phenolic groups content than did terrestrial HS. Some of these discrepancies were attributed to idiosyncrasies of the methodology, which seem to include other functions, such as the amide groups in the case of phenolic acidity. In order to discuss this point in greater depth, six standard samples (two FA and four HA) of known amino acid content, purchased from the International Humic Substances Society (IHSS), were also analyzed and the data were compared to recently published direct-titration data on the same set of samples.

ADDITIONAL INDEX WORDS: *Humic acids, fulvic acids, estuaries.*

INTRODUCTION

The dynamics and mechanisms of the interactions between humic substances (HS) and xenobiotics in natural environments depend basically on the HS functional groups content. Of these, the phenolic hydroxyls and carboxylic groups are considered to be the most important ionizable sites (SCHNITZER and GUPTA, 1965). Their concentration in HS depends on the organic matter (OM) source, the humification degree and also the environmental characteristics under which the HS were generated. In general, HS from marine environments have been shown to present lower phenolic contents than HS from terrestrial systems (RASHID, 1985). It has also been shown that HS from disturbed environments present lower carboxylic groups content than HS from preserved environments (KERNDORFF and SCHNITZER, 1979).

Studies on HS structural characteristics are particularly complicated because in addition to their natural complexity they have usually been obtained utilizing different methods, in different laboratories, inhibiting the comparison of results. Since the establishment of the IHSS procedure for HS collection (SWIFT, 1996), however, at least this part of the problem has been minimized and research on structural characteristics of HS has progressed considerably. Even so, most studies have been focused on terrestrial, mainly soil, material, a knowledge of properties of HS from aquatic environments being less developed.

With the purpose of improving our understanding vis-à-vis the structural and functional properties of HS from aquatic systems as well as of determining the relationships between such properties and the sources and evolution of OM within these environments a set of fulvic (FA) and humic acids (HA) extracted from marine, estuarine, lacustrine and terrestrial environments were obtained. The method utilized to extract the HS was that recommended by the International Humic Substances Society (IHSS). Structural and spectral properties of the samples were extensively analyzed by distinct methods (GIOVANELA *et al*., 2003; SIERRA *et al*., *submitted*) and the data supported both the idea that HS from aquatic and mixed environments are more aliphatic and have higher nitrogen, sulphur and oxygen contents than had previously been proposed and, also, that amide linkages form important fractions of their structures.

General data relating to the structural properties of aquatic HA are not abundant in the literature, and data on their acidity contents are even scarcer. The purpose of this study is to present acidity values for the above-mentioned set of samples, enriching the literature on HS studies, in this specific area of interest. A peat sample and a commercial HA were also included in this study, as examples of terrestrial materials.

METHODS

The coastal marine sediment samples were collected from Mar Virado Island (MVI) and Ubatumirim Beach (UBM); estuarine and lacustrine samples from Ratones Mangrove (RME) and Peri Lagoon (PLN) and; terrestrial environments were represented by a peat sample from Arroio of Silva Peat (ASP), and by the commercial Aldrich HA. The four sampling sites are located in the southern coastal zone of Brazil, the first two being in São Paulo and the other three in Santa Catarina. The Ratones Mangrove estuarine sediment samples were taken from distinct points within the estuary : a) the sea bottom, within the bay (RME G1 and RME G2); b) the shore near the sea (RME 1A) and; c) sites close to terrestrial vegetation, relatively far from the sea (RME 2A and G7). Only occasionally during exceptionally high flood tides does the RME 2A site inundate and receive marine inputs. Peri Lagoon is a freshwater lake. Three streams flow into this system, which is located in a hydrographic basin surrounded by a dense sub-tropical and well-preserved rain forest. Two samples were collected at two different points from the bottom of the lake: near to the edge (PLN 4) and in the middle of the lake (PLN 7).

All samples were obtained via the IHSS recommended procedure (IHSS home page, 2003). The Aldrich HA was submitted to the same purification process applied to the other samples (GIOVANELA *et al*., 2003).

Since the comparison of our data with previously published work was desirable the SCHNITZER and GUPTA (1965) method, which is often used in determinations of the concentrations of carboxylic and phenolic groups in HS, was chosen. This method employs ion exchange reactions, with Ca(CH3COO)2, to determine carboxylic acidity (CA) and with Ba(OH)2, to determine total acidity (TA). The difference between these

two values is then ascribed to phenolic acidity (PhA). Except when indicated all determinations were carried out in triplicate.

RESULTS AND DISCUSSION

The acidity values of the HS studied, as determined by the SCHNITZER and GUPTA (1965) method, are presented in Table 1. In spite of the heterogeneous nature of the material, standard deviation values are low, attesting the reproducibility of the measurements. The experiments with the Aldrich HA, for example, were carried out nine times, each time yielding approximately the same value. FA data were more reproducible than HA data, which could be ascribed to the fact that they were homogenized through adsorption-desorption onto XAD-8 resin. HA, on the other hand, underwent less modification during extraction and purification procedures, retaining more signals and impurities of the source material. Interestingly, however, for HA samples the CA does not vary very much, being between 3.07 and 5.66 meq g-1. For FA the range is higher: from 3.73 to 7.16 meq g-1. In the case of the TA values these trends are inverted, i.e. for HA the range of values is higher.

Comparing samples from the same parent sediment, FA, in general, had more acidic groups than HA, the only exception being the TA of the PLN 7 samples. The TA values determined here are, in general, higher than those reported for HS from distinct environments employing the same principles applied here (SCHNITZER and GUPTA, 1965; GILLAM and RILEY, 1982; EL-SAYED *et al*., 1996; MASINI *et al*., 1998; RITCHIE and PERDUE, 2003). RITCHIE and PERDUE (2003) compiled data from 284 studies which utilized the SCHNITZER and GUPTA method and showed that both, the phenolic and the carboxyl contents of HS ranged from 1 to 10 meq g-1 in all these estimates. Our CA ranged from 3.73 to 7.16 meq g-1 for FA, and from 3.07 to 5.66 meq g-1 for HA being, consequently, mid-range values. Our PhA, on the other hand, ranged from 7.72 to 16.12 for FA and from 7.34 to 20.34 meq g-1 for HA being, in most cases, higher than the highest values reported. The relatively high total acidities measured here may be, hence, a consequence of the high phenolic content.

These samples were from highly productive environments, with considerably high sedimentation rates and, these high acidities could, initially, be attributed to their low humification degree. In fact, from their elemental and spectral properties (GIOVANELA *et al*., 2003; SIERRA *et al*., submitted) it is observed that they preserve significant moieties of the source materials. Comparing samples from distinct sites, however, theacidity data seem to be in disagreement with the elemental and infrared properties. Algal biomass is aliphatic and proteinaceous in nature and the HS originating from it would be expected to be low in phenolic constituents when compared to terrestrial HS, which in general, have lignin moieties. For our samples both, carboxylic and phenolic contents, do not show specific trends, with the terrestrial samples in some cases exhibiting more carboxyl groups than the aquatic samples and the marine and lake samples presenting higher phenolic contents than the estuarine and terrestrial samples. Among the HA from aquatic environments, for example, the highest phenolic content is presented by a lake sample (PLN 7), which did not show phenol signals in its 13C-NMR spectrum (SIERRA *et al*., submitted). Considering the information obtained so far from this set of samples, and the reproducibility of the acidity data, we believe that these discrepancies are related to idiosyncrasies of the analytical methodology, as will be discussed below.

Acidity values obtained with the SCHNITZER and GUPTA (1965) method have often been unreliable mainly because acidic groups other than carboxylic and phenolic groups are presumed to interfere with the measurements (HOLTZCLAW and SPOSITO, 1979; PERDUE, 1980; RITCHIE and PERDUE, 2003). SCHNITZER and GUPTA (1965) even advised that the word phenolic should be placed within quotation marks whenever this method is used to determine phenolic contents in HS.

Table 1. *Acidity contents of humic substances (in meq g-1). *Standard deviations for nine replicates.*

Sample	CA	PhA	TA
FULVIC ACIDS			
MVI	4.63 ± 0.22	13.20 ± 0.79	17.83 ± 0.76
UBM	3.73 ± 0.04	15.27 ± 0.51	19.00 ± 0.50
PLN 4	4.70 ± 0.08	12.80 ± 0.51	17.50 ± 0.50
PLN 7	7.16 ± 0.01	7.72 ± 0.20	14.88 ± 0.20
RME 1A	5.13 ± 0.20	11.37 ± 0.54	16.50 ± 0.50
RME 2A	5.56 ± 0.02	13.11 ± 0.29	18.67 ± 0.29
PEAT D	6.21 ± 0.04	16.12 ± 0.29	22.33 ± 0.29
HUMIC ACIDS			
MVI	3.68 ± 0.04	11.25 ± 0.49	14.93 ± 0.49
UBM	3.17 ± 0.02	12.83 ± 0.50	16.00 ± 0.50
PLN 4	3.21 ± 0.06	12.29 ± 0.50	15.50 ± 0.50
PLN 7	3.77 ± 0.07	14.73 ± 0.50	18.50 ± 0.50
RME G1	3.07 ± 0.40	8.13 ± 0.50	11.20 ± 0.30
RME 1A	3.70 ± 0.06	9.63 ± 0.29	13.33 ± 0.29
RME G2	3.16 ± 0.40	7.34 ± 0.64	10.50 ± 0.50
RME G7	3.52 ± 0.16	14.48 ± 0.52	18.00 ± 0.50
RME 2A	3.74 ± 0.18	12.76 ± 0.53	16.50 ± 0.50
PEAT D	4.76 ± 0.18	13.24 ± 0.53	18.00 ± 0.50
PEAT F	5.66 ± 0.05	20.34 ± 0.50	26.00 ± 0.50
ALDRICH*	4.17 ± 0.03	8.83 ± 0.50	13.00 ± 0.50

Recently we tested the suitability of this method for weak-acid model mixtures both in the presence and absence of peptides. From such investigations we concluded that: (i) for the acid mixtures in the absence of peptides, certain substituted phenols with high pKa values, are only partially quantified; (ii) in the presence of competing basic sites like amine groups, the weak base Ca(CH3COO)2 can only partially deprotonate the carboxylic groups. These "ignored" carboxylic protons are, conversely, detected via the reaction with the strong base Ba(OH)2 being, consequently, accounted for in the phenolic content; (iii) furthermore, under the severe conditions of the reaction with Ba(OH)2, the hydrolysis of the amide linkages takes up extra hydroxyls, distorting the TA estimation. As the PhA is calculated from the difference between TA and CA these extra hydroxyls can be also accounted for in the phenolic content (SIERRA *et al*., in press). Based on these last two effects one could presuppose that HS with high amino acids content could present high "phenolic" acidity, whenever the SCHNITZER and GUPTA (1965) method is employed. The high nitrogen content as well as the presence of amide groups in our marine and lake samples could, in that case, be at the origin of the high phenolic content measured here.

To estimate the extent of such interference we determined, using the SCHNITZER and GUPTA (1965) method, the acidity of six IHSS samples of known amino acids content (IHSS home page, 2003). These data are shown in Table 2. The PhA values encountered for these samples are again very high ranging from 11.35 meq g-1 for the Elliot Soil HA to 21.07 meq g-1 for the Pahokee Peat FA. Nevertheless, contrary to expectation, the PhA does not correlate to the amino acid content of the samples. On the contrary, the Elliot soil HA for instance, exhibits the lowest PhA and the highest amino acids content. These results indicate then that the amide linkages are not the most important factor interfering with phenolic acidity measurements of HS by indirect titrations. Another constraint, the hydrolysis of ester groups, for example, has also been considered as a source of errors in HS acidity determinations (BOWLES *et al*., 1989; LEENHEER *et al*., 1995) and the possibility of the two effects occurring simultaneously cannot be disregarded.

Methodology Considerations

The functional groups content, as measured by direct potentiometric titration, of the same set of IHSS samples studied here has been recently published (RITCHIE and PERDUE, 2003) and, in Table 2 the data obtained with the two approaches can be compared. The PhA contents measured by direct potentiometric titration are much lower than those measured via indirect titration. As discussed above, it is not possible to know whether indirect titrations incorporate non-phenolic groups or direct titrations underestimate the phenolic content but, from these data, it becomes clear that the two methods measure different things. The bias which could be introduced by the hydrolysis of peptide linkages or ester groups in the alkaline pH range, for example, could be minimized under the conditions of the RITCHIE and PERDUE experiments, *i.e.*, rapid titrations to final pH values of 10.5-10.7.

Direct-titration carboxyl contents, on the other hand, were much higher than those measured via the SCHNITZER and GUPTA (1965) method. In the previously cited experiment with model compounds (SIERRA *et al*., *in press*), we observed that in the presence of competing basic sites (such as amine groups), the acetate ion does not displace all carboxylic protons, lowering the CA detection. The sodium hydroxide, customarily employed in direct potentiometric titrations, on the other hand, is a strong base displacing more efficiently the protons from a great number of sites. It should also be emphasized that RITCHIE and PERDUE (2003) reported the highest estimates of CA and the lowest estimates of PhA for fulvic and humic acids, when compared to other published direct-titration models (MILNE *et al*., 2001; SIERRA *et al*., 2001; GUSTAFSSON, 2001).

Interestingly, however, there is a manifest relationship between the CA obtained via the SCHNITZER and GUPTA (1965) and the RITCHIE and PERDUE (2003) approaches. For all samples, the CA, as determined by direct titration, is around twice the value determined via indirect titration. Such regularity is quite surprisingly and may indicate that the ratio between the abundance of strongly acidic carboxyl groups (those sufficiently acidic to exchange with the acetate ion) and total carboxyl groups (those detected via direct titration with a strong base) is constant for these samples. These data should, however, be interpreted with caution, since, as in the case of the indirect-titration method, the acidity values so obtained cannot be considered as definitive or absolute values because they are dependent on the model chosen to better fit the experimental data as well as on the procedural conditions (*e.g.* rapid or slow titration, high or low HS concentration, forward or backward titrations and so on). Such discrepancies confirm again that different approaches will produce different results for the same samples and reinforce the importance of the standardization of methodologies in HS studies.

As for our HS, and contrary to usual presumptions, the general order of the acidity content of the IHSS standard samples is also quite unsystematic. Perhaps the presupposed "trends" in the functional constitution of HS from distinct environments do not exist in real systems. In fact, HS are complex materials and differences observed among them may be related not only to the source-specific influences but also to the mechanisms of humus genesis, including the bacterial metabolism typical of the specific environment, the degree of humification and the physico-chemical characteristics of the surrounding areas.

CONCLUDING REMARKS

Acidity contents of a set of marine, estuarine, terrestrial and lake fulvic (FA) and humic (HA) acids were determined via the Ca-acetate and the Ba-hydroxide indirect-titration methods. Whereas the elemental and infrared properties of the samples clearly reflected the origin (aquatic or terrestrial) of the samples, the acidity contents were quite unsystematic with terrestrial samples exhibiting more carboxyl groups than aquatic samples and marine and lake samples presenting higher phenolic contents than terrestrial HS. These "discrepancies" were, at first, attributed to idiosyncrasies of the methodology, which seem to include other functions, such as amide groups, for instance, in the determination of phenolic acidity. To address this question, six IHSS standard samples of known amino acids content, were analyzed via the same procedure. Contrary to expectation, acidity did not correlate with the amino acids content discarding the possibility of amide linkages being chiefly responsible for the high phenolic content of samples from aquatic environments.

As for our samples, the acidity data for the IHSS samples did not follow any specific trend, even when measured via direct-titration (this latter information taken from the literature). These data raised some questions that are perhaps far from being answered: - Does the HS acidity really follow some kind of tendency (e.g., decreasing phenolic content and increasing carboxyl content moving from terrestrial to aquatic environments)? - Even if such a trend exists in nature, does it remain in the extensively handled (through usual extraction and purification procedures) FA and HA samples?

As a matter of fact the only reasonable way to compare functionalities of HS from distinct environments is by using standardized methodologies. In this concern the SCHNITZER and GUPTA (1965) method seems, in spite of the inexactitudes discussed here, to be, currently the more suitable. Firstly, because it is rapid and reproducible and can be carried out even in modest research laboratories. Secondly, notwithstanding the acidity values determined in this way not being of absolute significance, the acidic groups so detected are equally important as they are capable of carrying out the ionic exchange with the Ca-acetate and Ba-hydroxide reflecting therefore the reactivity of each sample. Whether or not the acidic groups so detected are "carboxylic" and "phenolic" is of no significance, and to avoid discussion on this, the expressions "Strong acidic

Table 2. *Acidity (in meq g $^{-1}$) and amino acids compositions (in μ mol g^{-1}) of the IHSS samples. [1]RITCHIE and PERDUE (2003); [2]IHSS home page (2003). Data without standard deviation are from a single measurement.*

Sample	CA (This work)	CA[1]	PhA (This work)	PhA[1]	TA (This work)	TA[1]	Total amino acids[2]
FULVIC ACIDS							
Suwannee River (1S101F)	5.15 ± 0.05	11.44 ± 0.10	14.99 ± 0.06	2.91 ± 0.10	20.14 ± 0.04	14.35 ± 0.14	24
Pahokee Peat (1S103F)	5.93 ± 0.08	13.34 ± 0.10	21.07 ± 0.51	2.32 ± 0.10	27.00 ± 0.50	15.66 ± 0.14	166
HUMIC ACIDS							
Suwannee River (1S101H)	4.86	9.59 ± 0.07	18.67	4.24 ± 0.07	23.53	13.83 ± 0.10	89
Pahokee Peat (1S103H)	4.46 ± 0.01	9.01 ± 0.09	16.79 ± 0.27	1.91 ± 0.09	21.25 ± 0.25	10.92 ± 0.13	373
Elliot Soil (1S102H)	3.90 ± 0.05	8.28 ± 0.09	11.35 ± 0.25	1.87 ± 0.09	15.25 ± 0.25	10.15 ± 0.13	777
Leonardite (1SI04H)	3.32 ± 0.02	7.46 ± 0.05	19.93 ± 0.29	2.31 ± 0.05	23.25 ± 0.25	9.77 ± 0.07	11

groups" and "weak acidic groups" could be used instead. A few points such as, the type of filter (PERDUE, 1980) and the quantity of samples used in each determination should, moreover, be standardized. The acidity values determined in this way should, however, have exclusively operational connotation, being used only for comparisons between HS receiving identical treatment.

ACKNOWLEDGEMENTS

This work was supported by the Conselho Nacional de Pesquisa e Desenvolvimento (CNPq, Brazil), the Fundação Nacional do Meio Ambiente (FNMA, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil).

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