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Chemical and physicochemical characteristics of humic acids extracted from compost, soil and amended soil

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Abstract

In order to gain understanding about how "HA-like substances" from organic amendments may change some properties in the soil solution, the knowledge of chemical and physicochemical characteristics (charge development, acid–base behavior and heterogeneity) should be known.

The aim of this research were (i) to study the elemental and functional composition, (ii) to determine charge behavior, acid–base properties (apparent dissociation constant and buffer capacity) and (iii) to evaluate heterogeneity of humic acids (HA) isolated from municipal solid waste compost (MWC) and from the corresponding MWC-amended soil, in comparison to those of the unamended soil HA using potentiometric titration and differential scanning potentiometry (DSP).

Potentiometric titration and the first derivative of $-Q$ versus pH (negative charge development versus pH) curves could be used to determine proton-affinity distribution and the chemical heterogeneity of the HA as well as the average pK_{app} and buffer capacity in a wide range of pH.

Differential scanning potentiometry allows determination of the pK_{app} values in a narrower range of pH than potentiometric titrations and is another simple methodology to study acid–base behavior of HA.

DSP allows us to determine seven different pK_{app} values for HA-S and HA-E and four different pK_{app} for HA-C. Each one of these values corresponds to known acidic groups that can be present in the macromolecule of HA.

Keywords: Humic acids; Potentiometric titration; Differential scanning potentiometry; Compost; Soil; Amended soil

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1. Introduction

Soil organic matter (SOM) is a basic component of the agroecosystem and acts as an essential link among the various chemical, physical and biological soil properties. It helps to prevent erosion and desertification and is a driving variable in environmental changes since it acts both as a source and as reservoir for carbon [1–3].

In recent years, the use of organic amendments, others than traditional manure, has become very popular and efficient for the improvement and/or restoration of SOM. Several organic residues and wastes produced by anthropic activities are potential candidates to recycling as soil amendment after appropriate treatments, which should be adequate to sanitation and reduction of metal and organic contaminants in the substrate, and transform the initial fresh organic matter (OM) into stabilized organic forms. These prerequisites are considered essential for any waste to be safely, conveniently and efficiently used as soil amendment in order to avoid or, at least, limit any hazard for chemical and biological soil properties, crop production and water quality.

Organic waste materials are rarely applied to soil in the fresh or raw state. Generally, they are properly processed previous to soil application, in order to obtain an amendment possessing beneficial rather than adverse effects on soil fertility and agricultural production. The most common treatment of organic raw materials is the composting process, which consists basically of a controlled biological transformation of raw OM operated by aerobic micro-organisms. During composting, a large part of the original OM is mineralized and the residual OM is transformed into new organic materials, in which the humification processes typically occurring under natural soil condition have started and proceeded to some degree, producing "humic-like substances". The composting makes the compost organic matter similar to soil organic matter [4–6].

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The OM is a heterogeneous mixture of vegetal, animal and microbial origin in various decomposition states. The main and more stable components of the OM are humic acids (HA) which interact with a wide range of substances, including metals, organic and inorganic pollutants in soils and waters [7,8].

Humic acids are complex and ill-defined polydispersed mixtures of heterogeneous polyelectrolytes, and behave as a weak polyelectrolytic acid and can be investigated by analytical techniques. Most of the acidity in the HA is due to the carboxylic, phenolic-OH and/or enolic groups which dissociate to develop negative charges. The imides groups (=NH_2), secondary and even tertiary amines, among others, can be protonated to develop positive charges at very low pH.

The degree to which these groups are ionized or protonated, at the environmental pH values, and their abundance and distribution influence the interaction between HA and soil contaminants [9].

One of the most important properties of the HA is its large buffer capacity in a wide pH range, which arises essentially from the dissociation of acidic functional groups of which they are particularly rich [10,11]. The exact contribution to the total soil buffer capacity is not well known; in general, soils rich in HA are well buffered [12]. The addition of composted material could contribute to the soil buffer capacity through their HA-like substances content; this issue together with their acid–base properties has been studied extensively in the last years [5,11,13].

In order to gain understanding about how “HA-like substances” from organic amendments may change some properties in the soil solution, the knowledge of chemical and physicochemical characteristics (charge development, acid–base behavior and heterogeneity) should be known [11,14,15].

The potentiometric titration of HA was used in previous papers, but the interpretation is a delicate problem due to the large variety of different functional groups and the heterogeneity of the macromolecule of HA [5,10,11].

Because of the randomness of formation, uniform behavior is not expected for this polydispersed product, and only trends can be predicted for the properties. Various intra- and inter-molecular interactions, which are dependent on solution condition, are expected for aqueous HA systems [16].

The aim of this research were (i) to study the elemental and functional composition, (ii) to determine charge behavior, acid–base properties (apparent dissociation constant and buffer capacity) and (iii) to evaluate heterogeneity of HA isolated from municipal solid waste compost (MWC) and from the corresponding MWC-amended soil, in comparison to those of the unamended soil HA using potentiometric titration and differential scanning potentiometry (DSP).

2. Experimental

2.1. Materials

The humic acids fraction from municipal solid waste compost, the corresponding MWC-amended soil and unamended soil were extracted with NaOH 0.1 mol L^{-1}, purified with HCl:HF (1:3) and dried at low temperature until constant weight, according to the procedure recommended by Chen et al. [17].

The HA samples used in this work were:

- HA extracted from an Entic Haplustol of Province of Cordoba, Argentina (unamended soil). Denoted as: HA-S.
- HA extracted from municipal solid waste compost. Denoted as: HA-C.
- HA extracted from MWC-amended soil after 9 month of treatment, at a rate of 20 t ha^{-1}. Denoted as: HA-E.

All solutions were prepared with tridistilled water and all the reagents were ACS reagent grade.

2.2. Methods

Ash contents were measured by heating the HA at 550 °C for 24 h. The elemental composition was determined by a C, H, N, S analyzer instrument Carlo Erba 1108, using isothiourea as standard. Oxygen was calculated by difference: $O\% = 100 - (C + H + N + S)\%$.

Total acidity and carboxylic groups (COOH) content was determined according to conventional methods described by Schnitzer and Gupta [18], and phenolic-OH group content was calculated by difference. All these determinations were performed in triplicate.

To obtain the E4/E6 ratio, the absorbance at 465 nm and 665 nm was measured using Spectronic 20 Genesys Spectrophotometer on solution of 3.0 mg of each HA in 10 mL of 0.05 mol L^{-1} $NaHCO_3$. The ratios of absorbance at 465 nm and 665 nm gave the E4/E6 ratio [19].

Potentiometric titrations were done according the technique proposed by Campitelli et al. [11]. It consisted briefly in the following steps: a stock of HA solution was prepared by dissolving HA with minimum volume of NaOH solution (0.1 mol L^{-1}) and adding water up to the final volume. An aliquot containing the desired amount of HA (≅7–8 mg) was transferred to the titration flask that contains 10 mL of tridistilled water. The titrant (HCl ≅ 0.05 mol L^{-1}) was added from an automatic burette (Schott Geräte T80/20) at a titrant rate of 0.1 ml/40 s. This rate was chosen taking into consideration that the variation of pH values should range between 0.02 and 0.04 pH units. The pH values were measured with an Orion Research 901 pH meter equipped with a glass combined electrode. All titrations were performed in absence of background electrolyte. A reference or blank titration was carried out for each titration curve in order to correct the HA charge development by dilution effect. Each problem solution was titrated at least three times, the reported data representing the average values. All the reagents were ACS reagent grade.

Differential scanning potentiometry was carried out according to the technique proposed by Ceppi et al. [10]. It consisted in: titrate a known mass of HA (≅3, 6 and 8 mg) in an aliquot (10 mL) of H_2SO_4 solution (approximately 0.025 mol L^{-1}) with NaOH (approximately 0.05 mol L^{-1}). A reference or blank titration was carried out for each sample titration in the following way: an aliquot (10 mL) of H_2SO_4 solution (approximately 0.025 mol L^{-1}) was titrated with NaOH (approximately 0.05 mol L^{-1}). All titrations were performed in absence of background electrolyte. NaOH and H_2SO_4 concentration were the same for all the experiments.

DSP was performed with an automatic burette (Schott Geräte T80/20) at a titrant rate of 0.1 ml/40 s. This rate was chosen taking into consideration that the variation of pH values should range between 0.02 and 0.04 pH units. The pH values were measured with an Orion Research 901 pH meter equipped with a glass combined electrode. Each problem solution was titrated at least three times, the reported data representing the average values. All the reagents were ACS reagent grade.

3. Results and discussion

The elemental analysis, H/C ratio, ash content and E4/E6 ratio of HA studied are shown in Table 1.

HA-C has higher N content than the other HA, probably due, to the raw material used in the composting process (30% manure, 40% municipal waste and 40% tree leaves).

According to Stevenson [12], the H/C ratio is believed to be a measure of the degree of the HA insaturation. This ratio for HA-C is higher than that obtained for other HA, indicating that HA-C has a great degree of saturation.

Kononova [19] suggests that the magnitude of E4/E6 ratio is related to the degree of condensation of the aromatic C network, with a low ratio indicative of relative high degree of condensation of aromatic humic constituents. Conversely, a high E4/E6 ratio reflects a low degree of aromatic condensation and allow to infer the presence of a relatively large proportion of aliphatic structure. Thus, HA-C has a high value of this ratio (Table 1), indicating a content of aliphatic chains greater than the other HA studied. HA-E and HA-S did not show great differences between them, only a slight diminution in the C content in HA-E with respect to HA-S. This could indicate that HA-C interacts with native HA and contributes to the accumulation organic C in soil [2,3,20].

The H/C and E4/E6 ratio of HA-E and HA-S indicate that the insaturation and aliphatic characteristics of both HA are similar, suggesting their association and/or interaction under natural conditions, after the compost application to the agricultural soil.

Total acidity, carboxylic and phenolic-OH groups of HA studied are shown in Table 2.

The acidic functional groups content (total acidity, carboxylic and phenolic-OH groups) of HA-C is lower than those corresponding to the other HA (HA-S and HA-E). This characteristic could indicate that HA-C are different from soil HA.

HA-E acidic functional groups content shows a slight diminution in comparison with HA-S after 9 months of the application of the composted material to the soil, indicating some interaction between both HA under natural conditions. These low-degree changes may be due to the short time in contact and/or the low ratio (20 t ha^{-1}) of compost used in the experiment to observe significant changes using these parameters.

Table 2
Acidic functional groups contents of HA studied (cmol kg^{-1})

HA sample	Total acidity	COOH	Phenolic-OH
HA-S	1161a	567a	594a
HA-C	698b	305b	393b
HA-E	1135c	550c	585c

Different letters (a–c) in the same column indicate significant differences ($p < 0.05$) according to Tukey test.

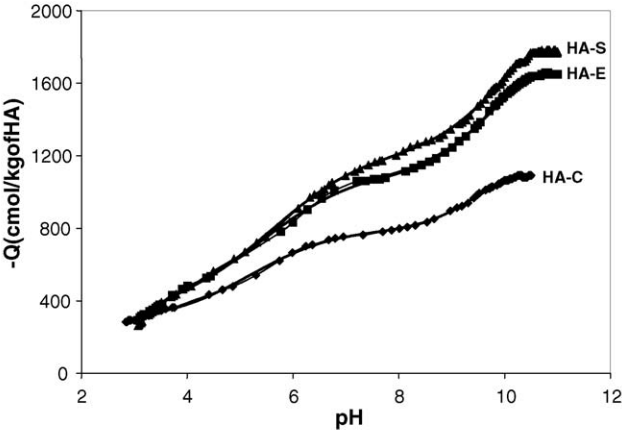

Fig. 1. Charge development of humic acids extracted from soil (HA-S), form compost (HA-C) and from amended soil (HA-E), in absence of background electrolyte. The solid lines were calculated on the basis of the sixth polynomial equation (with R^2 values exceeding 0.99 in all cases).

Charge–pH curves ($-Q$ versus pH) for all HA obtained from potentiometric titration curves (corrected for blank solution effect) are shown in Fig. 1. The solid lines are calculated based on sixth degree polynomial equation, according to the procedure proposed for Machesky [9] to fit the net experimental data (corrected for blank solution effect). The equation has the following form:

$$\text{cmol of H}^+ \text{ bound or release kg}^{-1} \text{ of HA } (-Q) = a(\text{pH})^6 + b(\text{pH})^5 + \cdots + f(\text{pH}) + g$$

This equation was used for smoothing purposes in the range of pH 3–10, with R^2 values exceeding 0.99 in all cases. In other studies, smaller order polynomial gave less acceptable fits and higher order polynomials resulted in no significant improvement [9,11].

Table 1
Elemental composition (ash and moisture-free basis), H/C and E4/E6 ratio of the HA studied

HA sample	C (%)	H (%)	N (%)	S (%)	O (%)	Ash (%)	H/C	E4/E6
HA-S	54.6	3.3	2.8	–[a]	39.4	1.70	0.71	4.18
HA-C	52.4	5.0	4.7	–[a]	37.9	1.27	1.14	6.84
HA-E	53.8	3.2	3.0	–[a]	40.0	3.98	0.71	4.30

[a] S content (<0.3%) detected but not quantified.

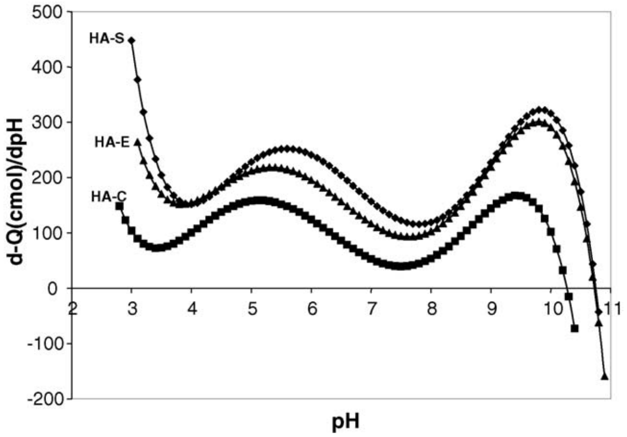

Fig. 2. Apparent proton-affinity distribution of the HA studied obtained from the first derivatives through charge–pH curves [d(−Q)/d(pH)] smoothing with sixth degree polynomial equation through the experimental data in the range of 3–11.

The charge development of HA-C was the lowest, in the whole pH range, probably due to the short and incomplete humification and oxidation process that takes place during the composting period and/or the raw material (municipal solid waste) used to obtain this compost.

The HA-E charge development was slightly lower than those observed for HA-S. This behavior could indicate the interaction between HA-S with HA-C after 9 month of compost application to the agricultural soil.

The differences observed in the charge development and the other parameters analyzed do not show great differences between HA-S and HA-E probably due to the low compost ratio used as an amendment (20 t ha^{-1}) and the short interaction time (9 month). However, these changes suggest some degree of interaction between HA-C and native HA (HA-S) after the compost application.

In this way, HA-S behaved as a sink for C from other sources like composted materials.

This interpretation agrees with other findings that support the hypothesis that the incorporation of product of exogenous degrading organic matter in the native humic material represents a basic mechanism of soil organic matter accumulation as well as the reason for its long-term stabilization [2,21,22].

The charge developments observed through this potentiometric titration are in concordance with those obtained with the methodology proposed by Schnitzer and Gupta [18] (Table 1).

The titration curves (−Q versus pH) show two inflexion points for all the HA studied. The first one, around pH 5–6 could be assigned, mainly, to carboxylic groups and the other, near pH 9, could correspond to other weak acidic groups like phenolic-OH and/or secondary or tertiary amines titrated together with phenolic groups.

The firsts derivatives of the −Q versus pH curves [d(−Q)/d(pH)] were obtained based on the smoothing function (sixth degree polynomial equation) through the experimental data [11,23,24]. These first derivatives curves represent apparent proton-affinity distribution of all the HA analyzed and are shown in Fig. 2.

When the affinity distribution shows adequately separated narrow peaks, the surface is characterized by a few discrete acids sites. The number of sites classes is then equal to the number of peaks and the affinity constants follow from the peak positions. When the distribution is wide and smooth, the surface is characterized as a continuous heterogeneous ligand [23–25].

We could separate the acidic functional groups into three sites domains: the strongest groups with $\text{p}K_{\text{app}}$ value around 3 assigned to *o*-COOH, the simple carboxylic groups (COOH) with $\text{p}K_{\text{app}}$ around 5.5 and the phenolic groups and other weak acidic groups with $\text{p}K_{\text{app}}$ around 9.5.

The characteristics of all the derivatives obtained for the different samples are similar. Distinct maxima and minima of [d(−Q)/d(pH)] are evident between pH 3 and 10 for the HA investigated in this study. The broad maximum occurring from 4 to 7.5 reflects the abundance and apparent proton-affinity distribution corresponding to the carboxylic functional groups. With this procedure it is difficult to identify different classes or sets of carboxylic groups regarding their $\text{p}K_{\text{app}}$ values; we can only observe the average $\text{p}K_{\text{app}}$ value.

The maximum around 5.5 observed for HA-C is wider and flatter than the maxima of HA-S and HA-E. This characteristic can indicate that in HA extracted from compost (HA-C) the probability to find sites with $\text{p}K_{\text{app}}$ values in the range pH + d(pH) is greater than those extracted from soil and amended soil and/or the number of these classes of acidic sites is lower than in the other HA analyzed. Thus, the heterogeneity of HA-C is higher than that observed in the other HA studied. This can be due to the different and incomplete humification process that takes place during the relatively short transformation periods in which composting is carried out. The characteristics (width and height) of the maxima around 5.5 for HA-S and HA-E suggest a low heterogeneity for these HA and/or the acidic functional groups content is greater than for HA-C, in this pH range.

The difference in the humification process can be seen, also, through the E4/E6 ratio (Table 1). This ratio can explain, in some sense, the heterogeneity of the HA studied. It is related to the degree of condensation of the aromatic C network [19]. Thus, HA-C has a large proportion of aliphatic structures and, in this way, has greater heterogeneity than the other two HA studied (HA-S and HA-E) according to that obtained by the first derivative of the charge development curve (Fig. 2).

The buffer capacity (β) of acid–base system is inversely proportional to the slope of the titration curves at a particular pH and is formally defined as:

$$\beta = \frac{\text{d}C}{\text{d(pH)}}$$

where dC is the number of moles/litre of strong acid or base required to produce a change in pH of d(pH).

Fig. 3 shows the buffer capacity (β) versus pH curves for three different mass values of HA-C. It can be noted that several β maxima can be obtained depending on the mass value.

The dependence of these β maxima at around pH 5–6 for all the HA studied, at the three different mass values analyzed is shown in Fig. 4. Under the concentration range studied (3, 6 and 8 mg), a linear trend was obtained; thus, it confirms the

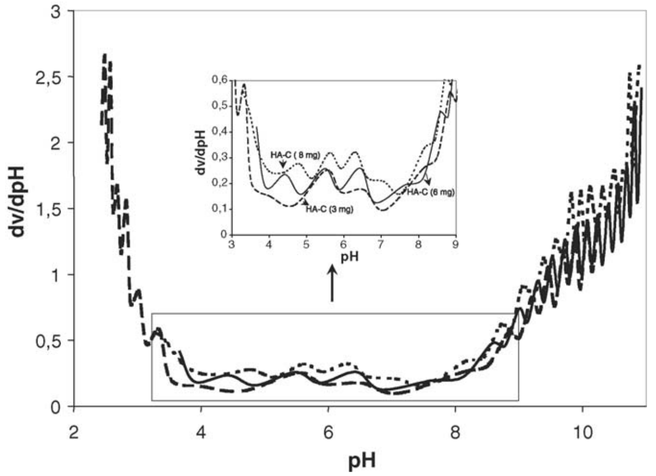

Fig. 3. Buffer capacity ($dv/d(pH)$) vs. pH curves for three different mass value of HA-C (3, 6 and 8 mg).

presence of a particular ionized functional group. This behavior validates the use of β for locating the HA buffer capacity and consequently the ionized acidic functional groups and their apparent dissociation constant values (pK_{app}).

Fig. 5 shows the first derivative ($dv/d(pH)$) of the net titration data (corrected for blank effect) obtained with differential scanning potentiometry.

This function is often used to locate buffer capacity maxima and minima for humic acids [9]. Buffer capacity distribution is also useful for modeling the acid–base properties of humic substances. Local maxima can be used to estimate the pH at which a particular acidic functional group is half-titrated [26]. Thus, each peak or maximum corresponds to a different set of acidic group and the apparent affinity constant follows from the peak or maximum position.

Distinct buffer capacity maxima and minima are evident between pH 3 and 10 for the HA investigated in this study (Fig. 3).

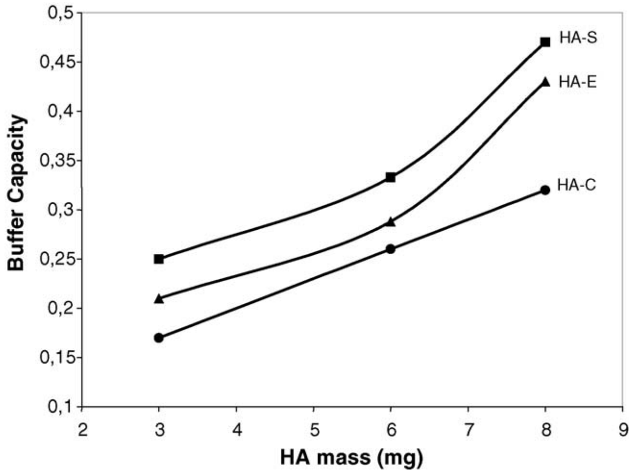

Fig. 4. The relationship between buffer capacity ($dv/d(pH)$) vs. HA mass (3, 6, and 8 mg) for all the HA studied (HA-S, HA-C and HA-E) at pH value 5–6 approximately.

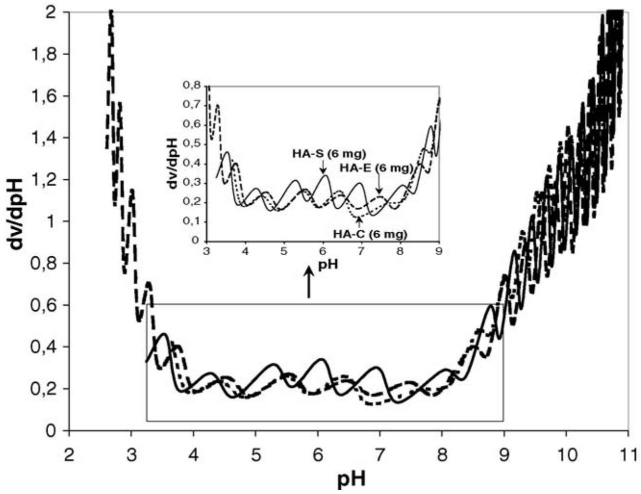

Fig. 5. The first derivative ($dv/d(pH)$) vs. pH of the net titration data (corrected for blank effect) obtained with differential scanning potentiometry (DSP), for HA-S: mass, 6 mg; HA-C: 6 mg; HA-E: 6 mg.

The broad maxima observed between pH 4 and 7.5, assigned to simple carboxylic groups, in Fig. 2, are divided in separate and distinct peaks for all HA studied (Fig. 5). For HA-S we can observe four different maxima, each one corresponding to a different set of this particular acidic group (COOH). The height of the maximum represents the abundance of this set of acidic group, and its contribution to the buffer capacity.

In the same pH range (4–7.5), HA-C has three peaks. Their height is lower than that observed for HA-S, indicating their lower content of these particular acidic groups (COOH), and their lower contribution to the buffer capacity. HA-E shows four peaks in this pH range, and their height is lower than HA-S but higher than that corresponding to HA-C. This behavior could indicate the interaction between HA-S and HA-C after the compost soil addition. The number and the height of the peaks observed in this pH range represent the content of these particular set of acidic groups (COOH). This is in agreement with the content obtained with the calcium acetate method (Table 2) and with that observed in Fig. 2.

At a pH value lower than 4, we can observe one defined peak for HA-S and HA-E but not for HA-C. This indicates that the first two HA have carboxylic groups with strong acid behavior (Fig. 5).

At pH values greater than 8, we can observe some different peaks. These peaks or maxima could be assigned to secondary or tertiary amines, probably titrated together with phenolic groups or other weak acidic functional groups [10,27–29].

The great number of peaks observed up to pH 9 could be due to some conformational changes produced when the macromolecule of HA becomes charged. At high pH values the functional groups are fully ionized and the charged groups try to locate as far as possible from each other. This behavior is difficult to explain at this high pH, but imbalance is a possibility [16,30].

Table 3 shows the values of apparent dissociation constant (pK_{aap}) of all the HA studied.

The apparent dissociation constant values can be assigned to different acidic functional groups K^1_{app}, K^2_{app}, K^3_{app} and K^4_{app}

Table 3
Values for the apparent dissociation constant (pK_{app}) of HA obtained by differential scanning potentiometry (DSP)

Humic acid	pK^1_{app} (K^1_{app})	pK^2_{app} (K^2_{app})	pK^3_{app} (K^3_{app})	pK^4_{app} (K^4_{app})	pK^5_{app} (K^5_{app})	pK^6_{app} (K^6_{app})	pK^7_{app} (K^7_{app})
HA-S	3.54 (2.88×10^{-4})	4.28 (5.25×10^{-5})	5.27 (5.37×10^{-6})	6.07 (8.51×10^{-7})	6.95 (1.12×10^{-7})	8.02 (9.55×10^{-9})	8.7 (1.99×10^{-9})
HA-E	3.76 (1.73×10^{-4})	4.53 (2.95×10^{-5})	5.53 (2.95×10^{-6})	6.46 (3.46×10^{-7})	7.47 (3.38×10^{-8})	8.48 (3.31×10^{-9})	9.00 (1×10^{-9})
HA-C	–	4.53 (2.95×10^{-5})	5.48 (3.31×10^{-6})	6.44 (3.63×10^{-7})	–	8.57 (2.69×10^{-9})	–

corresponding to carboxylic groups; K^5_{app}, K^6_{app}, K^7_{app} to secondary or tertiary amines titrated, probably, together with phenolic groups or other weak acidic functional groups. K^1_{app} value can be assigned to carboxylic sites like *o*-COOH which have a strong acid behavior.

It should be noted that the values of the apparent dissociation constant (K_{app}) obtained by differential scanning potentiometry are average values of apparent dissociation constant of a set of acidic sites involved in a range of pH narrower than that obtained by the method based on the first derivative [d($-Q$)/d(pH)], by which we obtain the apparent proton-affinity distribution.

4. Conclusions

HA-C has a greater aliphatic character, is less unsaturated, has less acidic functional groups content and has less charge development than the other HA analyzed.

Compost application as an organic amendment modifies, in some way, the chemical and physicochemical characteristics of HA extracted after 9 months of compost use. This behavior suggests the interaction between the native HA and "HA-like substances" present in the composted materials.

Potentiometric titration and the first derivative of $-Q$ versus pH curves can be used to determine proton-affinity distribution and the chemical heterogeneity of the HA as well as the average pK_{app} and buffer capacity in a wide range of pH. With this simple methodology it is possible to separate in three domains the acidic functional groups of the macromolecule.

Differential scanning potentiometry allows determination of the pK_{app} values in a narrower range of pH than potentiometric titrations and is another simple methodology to study acid–base behavior of HA.

DSP allows us to determine seven different pK_{app} values for HA-S and HA-E and four different pK_{app} for HA-C. Each one of these values corresponds to known acidic groups that can be present in the macromolecule of HA.

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References

[1] S.A. Boyd, L.E. Sommer, D.W. Nelson, Soil Sci. Soc. Am. J. 44 (1980) 1179–1186.

[2] A. Piccolo, R. Spaccini, G. Haberhauer, M.H. Gerzabek, Naturwissenschaften 86 (1999) 496–499.

[3] R. Spaccini, A. Piccolo, G. Haberhauer, M.H. Gerzabek, Eur. J. Soil Sci. 51 (2000) 583–594.

[4] N. Senesi, T.M. Miano, G. Brunetti, Humic-like substances in organic amendments and effects on native soil humic substaces, in: A. Piccolo (Ed.), Humic Substances in Terrestrial Ecosystems, 1996 (Chapter 14).

[5] J.C. Garcia-Gil, S.B. Ceppi, M.I. Velasco, A. Polo, N. Senesi, Geoderma 121 (2004) 135–142.

[6] R. Zbytniewski, B. Bussewski, Bio-Resource Technol. 96 (2005) 471–478.

[7] L.M. Yates, R.R. Engebreston, T.J. Haakenson, R. Von wandruszka, Anal. Chim. Acta 356 (1997) 295–301.

[8] J. Riggle, R. von Wandruska, Talanta 57 (2002) 519–526.

[9] M.L. Machesky, Sci. Technol. 27 (1993) 1182–1188.

[10] S.B. Ceppi, M.I. Velasco, C.P. De Pauli, Talanta 50 (1999) 1057–1063.

[11] P.A. Campitelli, M.I. Velasco, S.B. Ceppi, J. Chil. Chem. Soc. (2003) 91–96.

[12] F.J. Stevenson, Humus Chemistry—Genesis, Composition, Reactions, Wiley, NY, USA, 1994.

[13] M.J. Avena, L.K. Koopal, W.H. van Riemsdijk, J. Colloid Interface Sci. 217 (1999) 37–48.

[14] G. Sposito, K.M. Holtzclaw, D.A. Keech, Soil Sci. Soc. Am. J. (1977) 1119–1125.

[15] J.C. García-Gil Gallegos, tesis Doctoral Facultad de Ciencias UAM Madrid España, 2001.

[16] E. Tombácz, Soil Sci. 164 (1999) 814–824.

[17] Y. Chen, N. Senesi, M. Schnitzer, Geoderma 20 (1978) 87–104.

[18] M. Schnitzer, U.C. Gupta, Soil Sci. Soc. Am. Proc. 29 (1965) 274–280.

[19] M.M. Kononova, Materia Orgánica del Suelo, Vilassar de Mar. Barcelona, España, 1982.

[20] P. Buurman, The use of physico-chemical methods of analysis, in: P. Buurman (Ed.), Decomposition and Accumulation of Organic Matter in Terrestrial Ecosystems: Final Report, Ecosystem Research Report No. 1212, Commission of the European Communities, Bruselas, 1994.

[21] A. Piccolo, A. Zena, P. Conte, Commun. Soil Sci. Plant Anal. 27 (1996) 2909–2915.

[22] E. Lichtfouse, C. Chenu, F. Baudin, C. Leblond, M. Da Silva, F. Behar, Org. Geochem. 28 (1998) 411–415.

[23] J.C.M. de Wit, W.H. Van Riemsdijk, L.K. Koopal, Environ. Sci. Technol. 27 (1993) 2005–2014.

[24] J.C.M. de Wit, W.H. Van Riemsdijk, L.K. Koopal, Environ. Sci. Technol. 27 (1993) 2015–2022.

[25] M.M. Nederlof, J.C.M. de Wit, W.H. Van Riemsdijk, L.K. Koopal, Environ. Sci. Technol. 27 (1993) 846–856.

[26] B.A. Dempsey, C.R. O'Melia, in: R.F. Christman, E.T. Gjessing (Eds.), Aquatic and Terrestrial Humic Materials, Ann Arbor Science, Ann Arbor, MI, 1983.

[27] O.K. Borggaard, Acta Chem. Scand. 28 (1974) 121–122.

[28] J.C. Westall, J.D. Jones, G.D. Turner, J.M. Zachara, Environ. Sci. Technol. 29 (1995) 951–959.

[29] L.M. Aleixo, O.E.S. Godhino, W.F. Da Silva, Anal. Chem. Acta 257 (1992) 35–39.

[30] S.J. Marshal, S.D. Young, K. Gregson, Eur. J. Soil Sci. 46 (1995) 471–480.