

Comparison Between the Effect of Ionic Strength on Acidity and Dissociation Constants of Humic Acids Extracted from Sewage Sludge and Nile Water Hyacinth Composts

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Abstract: In this work humic acids were extracted from sewage sludge and Nile water hyacinth composts. The acids were investigated by chemical, spectroscopic and potentiometric analyses. The percent of carbon, hydrogen and nitrogen are in agreement with literature. HA_{ss} has higher percent of nitrogen which attributed to pertinacious structures. FTIR spectra of both acids exhibit, generally similar spectra with the typical major peaks reported for humic acids. Humic acid extracted from sewage sludge is characterized by lower average molecular weight, higher acidity, more phenolic groups and low heterogeneity than humic acids extracted from Nile hyacinth. The acidity of the two extracted humic acids were determined by potentiometric titrations and baryta method. The concentrations of COOH were found to be 2.90 and 3.74 meq/g H⁺ in HA extracted from sewage sludge and Nile hyacinth composts respectively, while the phenolic OH concentration was 4.86 and, 2.82 meq/g H⁺ in both acids respectively. The dissociation constant of COOH and phenolic OH acidic groups, pKa₁ and pKa₂ for the two acids were determined and effect of ionic strength on the ionization constants was studied and compared. In case of HA_{ss} both pKa₁ and pKa₂ generally decrease with increasing ionic strength. The overall intrinsic dissociation constant of HA_{ss} is found to be 16.3, while for HA_{NWH} they show no systematic dependence on the ionic strength. Accordingly, it was concluded that conformational changes may occur in these humic acids with the change in the suspension salt content, which was in agreement when compared with humic acids extracted from soil.

Key word: Humic acid • Acidity • Dissociation Constant • Effect of Ionic Strength

INTRODUCTION

Humic acids (HAs) are the most natural widespread complexing agents. They occur in soils, sediments and water as a product of the chemical and biological transformation of animal and plant residues. Recently, humic acids are produced for their environmental importance from many sources including composting of organic wastes. HAs have the ability to considerably affect mobility and availability of metals in the environment [1]. Humic acids are used to develop derived adsorbents, to remove heavy metal and organic contaminants from water [2]. The specific properties of humic acids products enable their application in industry, agriculture, environmental and biomedicine [3].

HAs are natural dark solid paramagnetic, macromolecular, heterogeneous substances with a high degree of polydispersity, extremely complex structure of

average molecular weight of 10³-10⁵ Da. They are known to contain similar functional groups such as C=C, COOH, OH, OCH₃, CO, or NH₂, arrangements such as redox quinone-semiquinon-hydroquinone, charge transfer planar complexes etc. The commonly encountered ionizable functional groups include carboxylic acids, phenols, alcohols, ammonium ions and thiols and to a lesser extent, sulfonic acids and "active methylene" compounds (with -CO-CH₂-CO- structural moiety) are also encountered [4]. The major binding sites of these functional groups are attributed to the oxygen-containing groups. These are carboxylic and phenolic groups, which contribute to the total acidity (TA) of humic substances. Phenolic groups are usually assumed to be the only weak acidic groups, while the contribution of alcoholic groups in carbohydrate entities and enols is usually neglected [5]. Due to the heterogeneity of the functional groups, polymeric nature and precipitation in acidic medium an

adequate methodology must be employed to titrate their acidities groups. Numerous methods of analysis for determination of HA oxygen containing functional groups have been published. Those methods include direct and indirect potentiometric titrations, thermometric titrations, nonaqueous titrations, infrared spectroscopy and ²⁹Si-nuclear magnetic resonance [4]. Potentiometric titration is frequently the method of choice for studying the acid-base properties of humic substances.

In this work two humic acids were extracted from sewage sludge and Nile water hyacinth composts. After purification, their basic characteristics are evaluated. The total acidity, contents of COOH and phenolic OH as weakly acidic function groups were determined by baryta and direct potentiometric titration methods. The effects of ionic strength on acidity and dissociation constants were studied and compared with the results published for soil humic acids. The discrete site model which means that the humic acid was treated as a mixture of monoprotic acids is assumed [6].

MATERIALS AND METHODS

Sample Collection: Nile water hyacinth plants which were mechanically collected from the water surface of the Nile in Alkkanater-AlKhayria area. It was accumulated in large heaps. The temperature of the heap was raised spontaneously with the evolution of very large amounts of water vapour. The heaps were stirred (to avoid firing) and then spread in the air under the direct sun light. Most of the plant parts including fibers were decomposed and changed into a material like clay species around plant stems which were remained. These masses of Nile Water Hyacinth Clay were collected and ground into a clay powder and used for humic acid extraction.

Sewage sludge samples are obtained from the drain station institute present in the area 9N of Alexandria city /Egypt and kept in plastic bags till use.

Humic Acids Extraction and Analysis: Extraction and purification of Nile Nile water hyacinth humic acids (HA_{NWH}) and sewage sludge humic acids (HAss), were carried out at 6hrs in absence of air according to IHSS (International Humic Substances Society Swift [7]. Drying, elemental analysis, moisture content, ash contents, determination of total acidity (carboxylic, and phenolic OH groups) by Ba(OH)₂ and calcium acetate method and E₄/E₆ (the ratio of absorbance at 400nm to absorbance at 600nm of sodium humate solution) were conducted as given by Stevenson [8]. Schnitzer and Vendette [9].

Potentiometric Measurements: Each of the two suspensions of HAss and HA_{NWH} was prepared by dissolving 0.03 g. HA in a minimum volume of 0.05 M NaOH solution in absence of air and reprecipitated by a suitable excess of diluted HCl. The suspensions transferred into a 50 ml volumetric flask with appropriate volumes of 1.00M NaCl to adjust the ionic strength at 0.01, 0.1, 0.2 and 0.4. Then the volume bringing up to the mark with distilled water. The whole volume was transferred into a titration vessel. Potentiometric measurements were made by using pH-meter (precision of 0.1 mV or 0.01 units of pH) using combination glass electrode (51935-00). Throughout the titrations an N₂ atmosphere was maintained by slightly bobbling through the solution, at room temperature. The titrations were performed against standardized NaOH solution. The pH was recorded after addition of equal increments (0.1ml. titrant) after constant time intervals of 30 second.

RESULTS AND DISCUSSION

According to baryta method the total acidity, or the sum of carboxylic plus phenolic groups, were determined by barium hydroxide, while the carboxylic acidity is determined by the Ca-acetate method. The concentration of phenolic groups is calculated as the difference between total and carboxylic acidity [8]. In the potentiometric titration method the total acidities can be evaluated by processing the titration data by the McCallum and Midgley's method [10], combined with Gran's method as described by Bizri *et al.* [11] and Brunelot *et al.* [12].

The data were processed according to relations 1 and 2:

$$(V_0+V) [H]^+ = f(V) \quad (1)$$

$$(V_0+V) [OH]^- = f(V) \quad (2)$$

Whereas

V₀ = Initial volume of the titrated sample contained humic acid suspension and the added strong acid.

V = Titrant alali volume added.

[H]⁺ = Free proton concentration;

[OH]⁻ = Free hydroxyl concentration.

Equation (1) is relative to the titration data before the equivalence point, while equation (2) is relevant after the equivalence point. The intercepts of the two lines intersecting the abscissa (V) allow to evaluate the two kinds of acidity i.e hydrochloric and humic acidities.

The titration curves of humic acids show no abrupt inflection points can be used to estimate the contents of carboxylic and phenolic groups. When the rate of the pH change ($\Delta\text{pH}/\Delta V$) is plotted against volume, a "multispiked" first derivative curve is resulted and the peaks of this curve should occur at the equivalence points of acidic functional groups [13]. The contributions of contents of COOH and OH groups to total acidity were determined by the aid of the differential curves in addition to simplified linear titration plots and Gran's plots.

According to Stevenson [8] and Senkyr *et al.* [14] and Choppin [15], it is possible to use the acidity values corresponding to –COOH and phenolic –OH groups, which are determined by standard methods for expressing humic acids concentration, thereby avoiding the problem of distribution in molecular weights.

Information about the acid dissociation constant (pKa) values of the acidic groups is necessary to calculate the pH dependent distribution of metal ions and metal humates. It is also a useful physicochemical parameter to control the metal binding abilities of humic acids. In this work the potentiometric titration data were used to calculate to calculate pKa1 and pKa2 for COOH and phenolic –OH functional groups respectively.

The Henderson-Hasselbach equation was used, [16]. It states that

$$\text{pH} = \text{pKa} + n \log \frac{A\theta + [\text{H}^+]}{A(1 - \theta) - [\text{H}^+]} \quad (4)$$

Whereas:

pKa is the apparent acid dissociation constant

A_t = Total content of weakly acidic or very weakly acidic functional groups.

θ = Degree of neutralization.

n = Corrector parameter of Henderson-Hasselbalch plot The relation between pH and log

$\frac{A\theta + [\text{H}^+]}{A(1 - \theta) - [\text{H}^+]}$ is plotted in. For θ = 0.5 the pH

corresponds to the pKa [16]. The calculations were made at different ionic strengths. The intrinsic dissociation constants were evaluated by extrapolating the pKa values to electrostatic free condition.

Chemical characteristics of H_{Ass} and H_{NWH}: Table (1) shows the basic characteristics of the two humic acids extracts. For agriculture application, the yield is

Table 1: Chemical characteristics of H_{Ass} and H_{NWH}(C,H,N% on dry ash free base)

Properties	H _{Ass}	H _{NWH}
Yield (in g/kg)	5.43	4.60
C%	50.15	50.32
H%	4.26	05.84
N%	9.05	04.29
O%	42.27	44.23
E ₄ /E ₆	4.92	4.301



Fig. 1: FTIR spectra of H_{Ass} (a) and H_{NWH} (a')

considered generally high [17]. The percent of carbon, hydrogen and nitrogen are in agreement with literature Stevenson [8], Rice and MacCarthy [18]. H_{Ass} has higher percent of nitrogen which may be due to pertinacious structures [19]. As E₄/E₆ was taken as indication of humic acids molecular weights where as it decrease with increasing of molecular weight H_{Ass} expected to have molecular weights lower than H_{NWH}.

Fig. (1) shows the FTIR spectra of H_{Ass} (a) and H_{NWH} (a'). Both acids exhibit, generally the similar spectra with the typical major peaks reported for humic acids. But H_{NWH} show more peaks than H_{Ass}. Which means that H_{NWH} has more functional groups and / or more heterogeneous than H_{Ass}. The similarity of the two spectra point to similar basic structures.

The presence of the ionizable function groups in the two acids is confirmed by the peaks at region 3367cm⁻¹ which are assigned to OH stretching vibration and possibly NH stretching vibration, for intermolecular hydrogen bonding and /or (H-bonded OH groups) attributed to phenolic group. –C=O stretching from –COOH (1700 cm⁻¹), overlapped with 1654 cm⁻¹ which could be attributed to aromatic C=C, C=O and/or C=O of conjugated ketones or to C=N amide I stretching. The band at 1230cm⁻¹ assigned to O-C-C ester linkage of carboxylic and phenolic acid, C–O stretch of COOH and CH₂ twisting. The band at 1033 cm⁻¹ due to orthosubstitution, C-C-O of primary alcohol [5, 20].

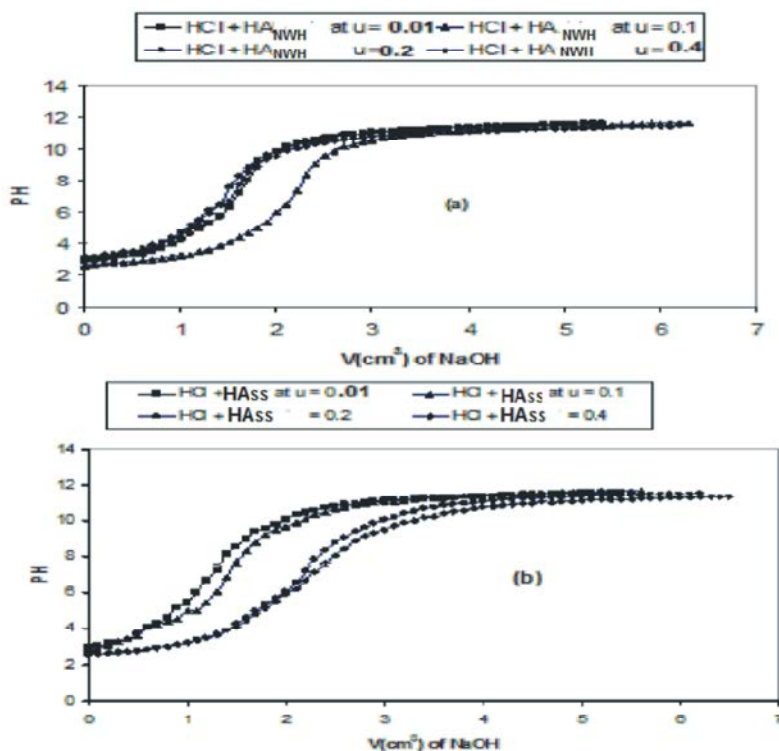


Fig. 2: Titration curve of HA_{NWH} (a) and $HAss$ (b) different ionic strengths.

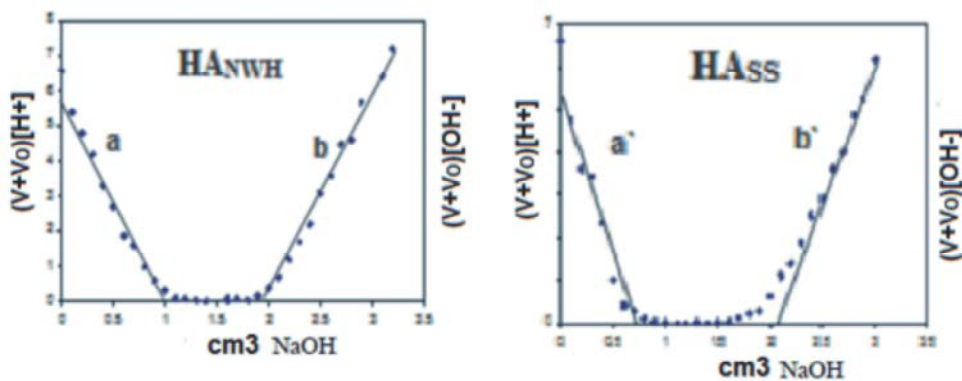


Fig. 3: Linear titration plots a, a' and Gran's plots b, b' for HA_{NWH} and $HAss$ as they are processed from data provided by titration of humic acid suspension in HCl against NaOH at ionic strength $\mu=0.01$ at $26^\circ C$

Potentiometric Determination Total Acidity, Contents of COOH and OH groups of $HAss$ and HA_{NWH} : Fig. (2) shows the titration curve of $HAss$ and HA_{NWH} under different ionic strengths. The absence of abrupt inflection points may be due to the presence of carboxylic and phenolic groups with different acidic strengths in addition to the electrostatic effect which appears due to charge accumulation on the humic acids macromolecules [21].

At titration of HCl-humic acids mixtures, against a standard NaOH solution, the amount of NaOH equivalent to the excess of HCl (V_s), can be determined from the

intersection of the straight line resulting from plotting $(V_o + V)[H^+]$ against cm^3 of NaOH added, the left hand lines in Fig. (3 a,a') (simplified linear titration plots), whereas the data processed were obtained from the lower pH region of the titration curve in each case.

At higher pH region of the titration curves, the plotting of $(V_o + V)[OH^-]$ against cm^3 of NaOH added gives the right hand straight lines (Fig 3, b,b') (Gran's plots). The intersections of the right hand lines with x-axis are equal to the amounts of NaOH required to neutralize HCl plus humic (V_e).

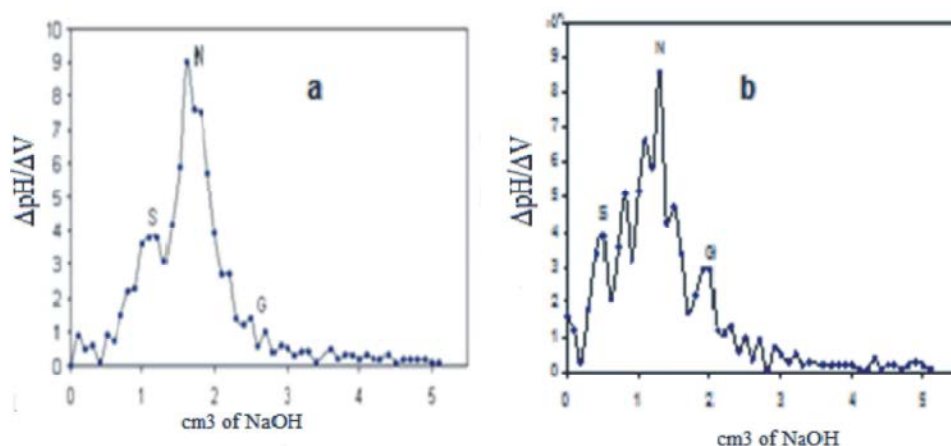


Fig. 4: First derivatives for titration curves of HA_{NWH} (a) and HA_{SS} at $\mu = 0.01$ at $C = 26^\circ$

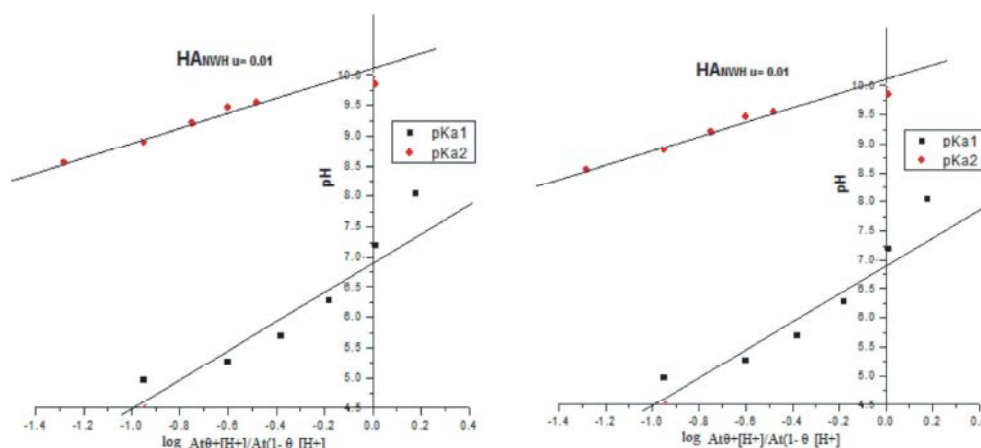


Fig. 5: Henderson-Hasselbalch plots for HA_{NWH} and HA_{SS} at $\mu = 0.01$, $t = 26^\circ C$

Fig (4) gives the corresponding differential curves (first derivative) of both humic acids titration curves at the same ionic strength. The point S of each differentiated curve represents the neutralization point of HCl. This was defined and confirmed with aid of Vs of linear titration plots.

The point G on the differential curves is the final end point of the titration and it is equivalent to V_e of Gran's plot. The difference ($V_e - V_s$) equivalent to the total titrable H^+ content of humic acid (total acidity). The point N is the apparent point of neutralization. The volume of the alkali at this point equal to that equivalent to the strong acid and the content of titrable H^+ ions in COOH groups of humic acid. Accordingly the volume equivalent to COOH groups ($V_{e_{COOH}}$). can be obtained. The volume of the NaOH titrant added from the point N to the point G, the final end point, will be equivalent to the content of very weakly acidic functional group ($V_{e_{OH}}$). The values of total acidity, contents of COOH and OH obtained by

calculation according to these principles for the two acids were processed in the same manner at ionic strength $\mu = 0.1, 0.2$ and 0.4 . The results obtained are shown in Table (2) with the corresponding values obtained by Baryta method.

The results showed no agreement between the values obtained for total acidity, by the barium hydroxide and potentiometric method in all cases. The values obtained by barayta method are higher than that obtained by potentiometric method. This result was explained by the impossibility of titrating all of the acidic groups in aqueous medium in the short time used in potentiometric method compared with baryta method where the solutions were shacked for 24hours. Also, according to the results HA_{SS} is more acidic and characterized by predominate of phenolic OH. Also the change of the ionic strength causes slight change in these amounts which differed according to the nature of humic acid and according to the type of the functional group.

Table 2: Total acidity, contents of COOH and OH groups of HAss and HA_{NWH} as determined potentiometrically under different ionic strength compared to the values obtained by Baryta method

Acidity in meq/g	Ionic strength of HA suspension							
	Hass				HANWH			
	0.01	0.1	0.2	0.4	0.01	0.1	0.2	0.4
T.A.	4.50	4.18	4.50	5.07	2.19	3.16	3.17	3.17
COOH	2.40	2.40	2.10	2.97	1.46	1.70	1.22	1.22
OH	2.10	1.78	2.40	2.10	0.73	1.46	1.95	1.95
Baryta method								
T.A	7.76				6.56			
COOH	2.90				3.74			
OH	4.86				2.82			

Table 3: The PH's of the starting point of neutralization (s), apparent point of neutralization (N) and final end point of neutralization (G) of HAss and HA_{NEH}.

μ	pH					
	Hass			HA _{NWH}		
	S	N	G	S	N	G
0.01	4.08	8.11	10.32	4.58	8.04	9.85
0.1	4.14	7.64	9.88	4.35	8.37	10.45
0.2	3.68	7.80	9.83	4.63	7.56	10.33
0.4	3.94	6.69	9.60	5.13	7.73	10.30

Table 4: PKa'S of HA_{SS} and HA_{NW} at different ionic strengths and Room temperature (t = 26°C)

μ	Dissociation constant					
	Hass			HA _{NWH}		
	pKa1	pKa2	pKa	pKa1	pKa2	pKa
0.01	6.45	9.74	16.19	6.75	10.10	16.85
0.10	5.25	9.55	14.80	6.50	10.30	16.80
0.20	5.34	8.72	14.06	6.90	09.20	16.10
0.40	5.15	8.58	13.73	6.70	09.80	16.50

These changes with ionic strength suggesting conformational changes may occur in these humic acids with the change in the suspension salt content [21].

Table (3) shows the PH's of the apparent point of neutralization (N) and final end point of neutralization (G) of HAss and HA_{NEH}. For both HAss and HA_{NWH} the COOH neutralized between (S) and (N) while the OH groups

neutralized between (N) and (G). According to these results, the acidic functional groups of HAss neutralized at slightly lower pH range than the corresponding HA_{NWH} groups. The average range approximately is 3.69–7.56 for HAss COOH and 7.56–9.9 for HAss OH groups. But 4.67- 7.93 and 7.93-10 .23 for HA_{NWH} COOH groups and HA_{NWH} OH groups respectively.

Pka'S of HA_{SS} and HA_{NW} at Different Ionic Strengths:

The calculations pK_{a1} and pK_{a2} were made according to Henderson-Hasselbalch equation. By using carboxylic acid groups concentration only to find pK_{a1} at each of the conditions studied. The required data were obtained by analyzing potentiometric titration curves at the pH range of neutralizing the carboxylic acid groups. Similarly pK_{a2} of OH groups were calculated on the same bases. The corresponding Henderson-Hasselbalch plots for both HAss and HA_{NWH} at $u=0.01$ -as an example-, are given in Fig (4). The pka's of HAss and HA_{NWH} at different ionic strengths are calculated and summarizes in Table (4).

For HAss, the effects of ionic strength on the dissociation properties are more pronounced. The effects appear on in the pK_{a1} values of the titratable COOH groups, which is larger at ionic strength 0.01, tend to be minimized with the increase in the ionic strength. The effect on pK_{a2} values of the titratable OH groups do not show these trend although it also decrease with increasing ionic strength. This trend reflects the expected effect of ionic strength on apparent dissociation constants as a function of the macro-ionic characteristics of the humic acid macromolecules [22]. For HA_{NWH}, although the results indicate a significant influence of electrostatic effects on the protonation behavior, the pK_{a1} and pK_{a2} values of HA_{NWH} show no systematic dependence on the ionic strength. This complication may due to the heterogeneous nature with respect to the dissociating groups and molecular size.

In Table (5) The pH ionization range, pKa's and the corrector parameter of Henderson-Hasselbalch plot n , of HAss and HA_{NWH} are compared with their corresponding results of three humic acids (HA1,HA2.and HA3) extracted from three soil under different plant conditions which were previously studied [23]. For both HAss and HA_{NWH} the decrease in pKa and the pH ionization range are generally similar with soil humic acids especially in case of carboxylic groups. Accordingly conformational changes may occur in the extracted humic acids with the change in the suspension salt content as well as soil humic acids . Also according to the pKa values, both HAss and are HA_{NWH} weaker acids than soil humic acids.

Table 5: Dissociation behavior of Hass and HA_{NWH} under the effect of suddenly rise in ionic strength in comparison with soil humic acids

Acid	u	pKa	
		pKa1 (pH range) n=	pKa1 (pH range) n=
Soil HA1	0.01	5.88 (4.91-7.91) n=0.33	9.24 (8.71-9.61) n=0.10
	0.10	5.30 (4.31-7.11) n=0.33	8.80 (7.71-9.11) n=0.14
Soil HA2	0.01	5.75 (4.91-8.11) n=0.38	9.40 (8.91-9.31) n=0.10
	0.10	5.10 (4.31-6.71) n=0.25	8.31 (7.31-9.11) n=0.33
Soil HA3	0.01	5.20 (4.31-6.31) n=0.25	8.52 (7.91-9.31) n=0.33
	0.10	4.90 (4.11-6.61) n=0.22	8.28 (7.31-9.26) n=0.33
HAss	0.01	6.45 (4.08-8.11) n=0.25	9.74(8.11-10.32) n=0.39
	0.10	5.25 (4.14-7.64) n=0.29	9.55 (7.64-9.88) n=0.33
HA _{NWH}	0.01	6.75 (4.58-8.04) n= 0.22	10.10 (8.04-9.58) n=0.66
	0.10	6.50 (4.55-8.37) n=0.25	10.30 (8.37-10.33) n=0.50

CONCLUSION

According to the results HAss is more acidic than HA_{NWH} and characterized by predominate of phenolic OH with respect to COOH groups. Conformational changes may occur in these humic acids with the change in the suspension salt content. For Hass also, pKa₁ and pKa show general decrease with increasing ionic strength. For HA_{NWH} protonation behavior show no systematic dependence on the ionic strength. This complication may due to the heterogeneous nature with respect to the dissociating groups and molecular size, but both HAss and are HA_{NWH} weaker acids than soil humic acids.

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