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FULVIC ACID-METAL ION INTERACTIONS IN WATER

by

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ABSTRACT

This report emphasizes the further characterization of fulvic and humic acids isolated from the B_2 horizon of a Podzol soil obtained at Conway, N.H., and isolated from the Oyster River (Lee, N.H.). We measured the E_4/E_6 ratios (absorbance ratio at 465 nm/665 nm) and the absorptivities of all the humic material samples.

We also describe the cryoscopic determination of the dissociation-corrected number-average molecular weights $\overline{Mn(corr)}$ of the soil and aquatic fulvic acid samples. The corrections for dissociation of fulvic acid were determined by a theory which utilizes the equivalents per gram and the acid dissociation constants of the fulvic acid samples. The soil and aquatic fulvic acid $\overline{Mn(corr)}$ values are 644 and 626, respectively.

We analyzed the solid state and aqueous solution electron spin resonances (esr) spectra of the aquatic and soil fulvic and humic acids. Because the aqueous solution esr spectra mimic the behavior of the model compound para-benzosemiquinone, we conclude that semiquinone free radicals predominate in fulvic acid. In addition a decrease in spin concentration at a potential of 0.20 volts (vs. SCE) demonstrates that the semiquinone radicals are at least partially responsible for the reducing capability of humic materials. From the above results we devised a quantitative semiquinone analysis for humic materials.

The results reported here in conjunction with our earlier studies on soil and aquatic humic matter are strong evidence for the similarity of humic materials isolated from soil and water. We emphasize the importance of simultaneous experiments on soil and water humic material.

INTRODUCTION

The separate occurrence of toxic metal ions or organic matter in the sources of domestic water is a cause for concern about water quality. When water supply sources are tested for toxic metals, the results are not totally comforting. Metal analyses of several United States rivers demonstrate that the mean cadmium value is near the maximum allowable United States Public Health Service standard and that the highest lead value is about three times the allowed value. Many sources of domestic water supplies contain excessive metal ion concentrations. Aquatic organic matter even in the absence of metal ions can also adversely affect water quality. For example organic matter is implicated in chloroform formation during the chlorination water treatment process. The removal of the organic matter is essential to make domestic water esthetically pleasing as well as potable (1).

It is apparent that the metal ions in organic-laden waters do not occur primarily as simple hydrated metal ions, because of discrepancies in physical and chemical properties. These properties are changed because of metal ion-organic interactions, that is by the formation of metal chelates. The water treatment problems are amplified when the metal ions and the organic matter are simultaneously present. As an example, the presence of metal chelates between iron and natural organic matter in natural waters make the removal of iron by oxygenation more difficult (2). A more serious water treatment problem occurs because of the well-known affinity of toxic metal ions like cadmium(II) and lead(II) for organic matter in water. The organic matter will very likely make toxic metal removal more difficult. In addition, the effect of toxic and

non-toxic metal ions on the formation of carcinogenic chloroform during chlorination of organic-containing waters is virtually unknown. The presence of metal ion-organic metal chelates might increase the rate or extent of chloroform formation.

Because of the importance of metal chelates in natural water chemistry, we are in the midst of an extensive study of water organic matter and its metal chelates. The goals of the research are all directed toward the understanding of metal ion-organic interactions in water and their effect on the removal of organic matter and toxic metals during water treatment processes. The results of this research will be useful to people studying water quality and water treatment processes. Our conclusions coupled with those of others might lead to more efficient processes for the removal of toxic metals and the prevention of chloroform formation during domestic water treatment.

The nature of humic materials in natural waters has been studied by several groups in the past ten years. Some of the more recent papers (3, 4) will serve as an entry into the older literature. In addition, two relatively recent books summarize the literature of humic matter. Gjessing has recently discussed aquatic humus (5), and the book by Schnitzer and Khan (6) represents an excellent review of soil organic matter research.

Although it has not been completely proven, we have much evidence that the "yellow organic acids" dissolved in water and the organic acids extracted from soils have approximately the same composition. Therefore, we will use the soil terms "fulvic acid" and "humic acid", to represent the water-derived organic acid fractions soluble and insoluble in water at pH 1, respectively. The term "humic materials" represents both the fulvic and humic acid fractions.

Our paper (7) on aquatic organic acids reported the isolation and characterization of fulvic and humic acids from the Oyster River (Lee, N. H.) and Jewell Pond (Stratham, N. H.), and fulvic acid from the B_2 horizon of a Podzol soil (Conway, N. H.). The soil organic matter was isolated by standard techniques (6, 8), but the water humic matter was isolated by a new process which involved the use of an ion exchange, Rohm and Haas XAD-2, and cation exchange resins. Ash tests demonstrated that the humic materials were low in inorganic compounds. The carbon, hydrogen, and nitrogen elemental analyses on the isolated humic materials showed that the soil and water fulvic acid samples are similar to each other, but that they are different from the two humic acid samples.

In this report we will discuss our continuing studies on the characterization of aquatic and soil fulvic and humic acids. We will include the isolation and characterization of soil humic acid as well as visible spectrophotometric, molecular weight, electron spin resonance spectroscopic, and reduction potential measurements of various humic materials.

EXPERIMENTAL AND CALCULATIONS

Materials

Common chemicals were used as purchased. The Fluka humic acid was purchased from Columbia Organic Chemical Co., Inc. (Columbia, South Carolina). It was purified by dissolving it in 0.01 M NaOH, centrifuging, and decanting off the solution. The pH of the solution was adjusted to pH 1 with HCl and the precipitated humic acid was separated and washed with water until free of chloride ion. The Podzol soil humic acid (B_2 horizon, Conway, N. H.) was isolated by a known method (6,8); the isolation and elemental and group analyses of the other humic materials were previously described (7).

The humic acids need additional treatment to decrease their ash content. In a typical procedure 10 g of humic acid were added to 400 ml of a solution containing 2 ml of 37.5% HCl and 2 ml of 49% HF. The solutions were stirred 1 hr at room temperature, filtered, washed until free of Cl^-, and air-dried in porcelin evaporating dishes in a hood. See TABLES 1 and 2 for the elemental, ash content, and functional group analyses of the humic materials.

Titrations of Fulvic Acid Samples with NaOH

In a typical experiment 0.02380 g of soil fulvic acid were diluted to 25 ml with aqueous 0.1M $NaClO_4$. Two 10 ml aliquots were withdrawn and diluted to 15 ml with 0.1M $NaClO_4$. Each aliquots was separately titrated with standardized 0.1M NaOH delivered with a Gilmont Micrometer Buret (model 7876). The pH measurements were performed using an Orion Model 407 Specific Ion Meter with a Corning Model 476050 combination pH electrode.

The titrations were done under N_2; 2 drops of 2-octanol were added to prevent foaming. The results are in TABLE 3. The calculations were performed according to Borggaard (9) using a computer program. Gamble (10) did a more detailed calculation.

Spectrophotometric Analysis of Humic and Fulvic Acids

The E_4/E_6 ratio, the absorbance ratio at 465 nm/665 nm, was obtained using a Cary spectrophotometer after dissolving the humic substances in 0.1M aqueous NaOH at concentrations of approximately 100 mg/l (TABLE 4). The absortivity in units of $ppm^{-1}cm^{-1}$ was obtained by dividing the absorbance at 465 nm by the concentration (ppm) of the humic material.

We also measured the absorbance as a function of pH. We used a three-necked flask containing a pH electrode, an N_2 inlet and outlet, and a serum cap. After lowering the pH to 1.6, we titrated a sample containing 11.38 mg of soil fulvic acid per 50 ml of solution (228 ppm) with 0.5M NaOH. The titration additions and the aliquot removals were done through the serum cap to insure anaerobic conditions. The data are shown in TABLE 5.

Attempted Measurements of Cu^{2+}/Fulvic Acid Chelation Ratio

We attempted to determine the Cu^{2+}/fulvic acid ratio in the Cu^{2+}-fulvic acid complex using spectrophotometric data and a Job's Method analysis. A soil fulvic acid sample solution was prepared which contained 3.6 meq of chelating sites per liter. A 3.6×10^{-3}M Cu^{+2} solution was also prepared. A series of 4 solutions was prepared according to the method of continuous variation. Each solution was 0.1M in $NaClO_4$ and was adjusted to pH 4.0 with NaOH and $HClO_4$. The mole fraction of Cu^{+2} varied from 0.2 to 0.8 in these

solutions. The spectra of the solutions were recorded in the visible range using the Cary 14 Model Spectrophotometer. The spectrum of a Cu^{+2} solution of corresponding ionic strength, pH, and concentration was also recorded; as were the spectra of several fulvic acid solutions of corresponding ionic strength, pH, and concentration. The Job's plot constructed from the data was too scattered to be of any significance. This was largely due to the small differences in absorbance between the ligand spectra and the complex spectra.

We then scanned wavelengths outside of the visible region in an attempt to find a wavelength at which the difference between the absorbance of the ligand and that of the complex is greater. The data used to construct a Job's plot were too scattered to be useful.

Part of the problem was in determining the absorbance of the fulvic acid solutions. Because the method depends upon the subtraction of the absorbance of the ligand from that of the complex and because in this case the difference was very small, it was necessary that the absorbance of the ligand solution be determined exactly.

Unfortunately, very small but significant deviations in fulvic acid absorbances were found and these may have enhanced the scatter in the plot. From this problem we concluded that a more exact method would be to do differential spectrophotometry. This can be done by using a complex solution in the sample cell and a pure ligand solution, of a concentration corresponding to the pure ligand concentration in the sample cell, in the reference cell. In this manner absorbances would be the result of complex formation. The following procedure was used.

A series of 10 solutions was made according to the method of continuous variation using 2×10^{-3}M fulvic acid and 2×10^{-3}M $Cu(NO_3)_2$

stock solutions. All solutions were made 0.1M in $NaClO_4$ and were adjusted to pH 4. In addition a set of fulvic acid solutions was prepared. The fulvic acid concentration in these solutions corresponded to the fulvic acid concentrations in the solutions made according to the method of continuous variation, assuming that no complex formation occurred. These solutions were also 0.1M in $NaClO_4$ and were adjusted to pH 4.

The Cary 14 spectrophotometer was used to record absorbances. Differential spectrophotometry was the method and the slide-wire was changed so that the measurement was in the abosrbance range of 0.0-0.2. For each Cu^{2+}-fulvic acid solution placed in the sample cell for a reading, the fulvic acid solution of corresponding concentration was placed in the reference cell so that absorbance change due to complex formation could be measured directly. The results are inconclusive.

We realized that it is not possible to determined the pure ligand concentration in the sample cell. In Job's Method the assumption is made that the ligand concentration is the same after complex formation, which is not true. Therefore it seems inappropriate to subtract the absorbance of a pure ligand solution of concentration equal to the concentration of ligand before complex formation from the absorbance of the complexed solution. This effect might explain the inconclusive results.

Molecular Weight Determination

The molecular weights were measured by a cryoscopic technique using the Advanced Instruments Model 600-5 widerange osmometer. Initially, the solution is supercooled to a temperature below its freezing point. The second step involves the agitation of the solution with a vibrator to initiate the crystallization of the water solvent. Third, the temperature

during the freezing process is measured to an accuracy of 0.001°C with a thermister. The instrument readout (Θ) is in units of the total millimolality of the particles present.

Molecular Weight Calculations

Glover (11) has written an excellent review on the determination of polymer molecular weights by freezing point depression and other colligative properties. The review includes experimental details as well as computational approaches. The instrument readout Θ can be related to the grams of solute per kilogram of solvent (W) by a power series in which $\underline{a}$ and $\underline{b}$ are constants (eqn. 1). The constant $\underline{a}$ is directly related to the number-average molecular

$$\Theta = \underline{a}W + \underline{b}W^2 + \ldots \quad (1)$$

weight ($\overline{Mn}$) by the apparatus constant K_{app} (eqn. 2). Thus once $\underline{a}$ of eqn. 1

$$\overline{Mn} = K_{app}/\underline{a} \quad (2)$$

and K_{app} have been determined, $\overline{Mn}$ can be easily calculated. Alternatively, one can multiply Θ by 1.856 to obtain the freezing point depression ΔT (°C x 10^3 units), and utilize it to calculate $\overline{Mn}$.

We utilized a second virial model for our calculations. That is we included the W and W^2 terms in our calculation of $\underline{a}$. The exact calculation we used is the zero point method (12), which corrects for non-zero Θ in the absence of a solute. In this method any zero point error is removed by subtracting W_1 and Θ_1 for the lowest concentration from each of the other values. Then using a least square analysis we calculated $\underline{a}$. Details of the calculation can be found in APPENDIX I. A plot of $\Theta-\Theta_1$ against $W-W_1$ is a graphical approximation to the solution which yields $\underline{a}$ as a slope.

The apparatus constant K_{app} depends on the molecular weight of the calibrating compound, the cryoscopic constant of the solvent, and other terms. The K_{app} value of 1004 °Cmolkg^{-1} was determined using sucrose. It was calculated via eqn. 2 from a and the 342.3 molecular weight of sucrose.

The acid dissociation equilibrium constants necessary for the calculations of model compounds are available (13). The acids (K values) are: ascorbic acid ($K_1 = 6.76 \times 10^{-5}$, $K_2 = 2.69 \times 10^{-12}$), succinic acid ($K_1 = 6.0 \times 10^{-5}$, $K_2 = 2.29 \times 10^{-6}$), tartaric acid ($K_1 = 9.12 \times 10^{-4}$, $K_2 = 4.26 \times 10^{-5}$), oxalic acid ($K_1 = 5.89 \times 10^{-2}$, $K_2 = 6.45 \times 10^{-5}$), and trimellitic acid ($K_1 = 3.02 \times 10^{-3}$, $K_2 = 4.47 \times 10^{-4}$, $K_3 = 6.3 \times 10^{-6}$).

The average deviation of observed and theoretical molecular weights of the model compounds is 2.4% (TABLE 6). The typical error in the calculation of $\overline{Mn(corr)}$ for a fulvic acid sample at the 95% confidence level is ±5%. In replicate analyses the standard deviation of the soil or water fulvic acid $\overline{Mn(corr)}$ values from their means is 20. The experimental data for the $\overline{Mn(corr)}$ calculations are in TABLE 7.

Electron Spin Resonance (esr) Experiments

For esr measurements solutions of previously dried 0.2 g fulvic acid samples were made up in 25 ml volumetric flasks, and were shaken for 24 hr. Then 4 ml aliquots were transferred to serum-capped vials, and the solutions were rendered anaerobic by repeated evacuate-freeze-thaw cycles under N_2. The pH was adjusted at room temperature with an anaerobic 2M NaOH solution in a N_2-filled glove bag. Solutions were introduced into the N_2-purged esr flat cell via a syringe and the room temperature spectra

were recorded by a Varian E-4 EPR spectrometer. A 1.00×10^{-4}M Fremy's salt solution was used to calibrate the signal area. The solid state samples were run on a Varian E-9 EPR spectrometer at room temperature. The signal areas were obtained by the first moment method (see APPENDIX II).

Controlled Electrolysis Experiments

The anaerobic controlled potential electrolysis was carried out by a Princeton applied Research Model 174A polarographic analyzer. The cell consisted of a calomel reference electrode and two platinum foil electrodes. Samples at pH 11.2 were withdrawn periodically and injected into the purged esr flat cell. Replicate analyses showed an average deviation of $\pm$ 5% of the measured signal. A nonelectrolyzed sample showed no decrease in esr signal during the time of the experiments.

Measurement of Reduction Potentials (E_h) of Fulvic Acids

The Orion model 96-78 combination redox electrode was used in conjunction with an Orion model 407 Specific Ion Meter. The system was calibrated by Zobell solutions. The anaerobic measurements were carried out in the cell described in the Spectrophotometric Analyses of Humic and Fulvic Acids section.

The E_h-pH experiments were done on solutions of 0.05, 0.10, and 0.15 g/25 ml concentration under N_2. To prevent foaming due to the bubbling N_2, 2 drops of octanol were added to the solutions. As the NaOH increments were added, the E_h and pH values were measured. The measured potentials were corrected for the SCE electrode; the E_h and pH values were also corrected for volume changes. The data shown in TABLE 8 were extrapolated to pH 0 by a linear

regression program (correlation coefficient, 0.98). The addition of air during another set of measurements had negligible effect of the E_h values.

RESULTS AND DISCUSSION

Isolation, Visible Spectra, and Functional Group Analyses

In a recent study (7) we isolated humic and fulvic acids from soil by a known method (6,8), and fulvic and humic acids from water by several methods including a new method involving anion exchange, cation exchange, and molecule-absorbing resins. The new XAD-2 isolation procedure has several advantages for the large scale isolation of humic materials from water. (a) Both the XE-279 and XAD-2 resins are commercially available in large quantities at moderate cost. (b) Neither of these resins, unlike most othr resins, is readily fouled by organic matter. That is, there is no artificial fractionation because some of the humic materials cannot be desorbed from the resin. (c) We avoided the use of organic reagents. Because of the fantastic adsorbing capability of humic materials (6), one can never be sure that they are completely removed.

The use of the XAD-2 resin is the key to the new isolation process. This hydrophobic polystyrene resin adsorbs molecular solutes by Van der Waals forces. Burnham *et al.* (15), for example, observed that the XAD-2 resin can be used to isolate phenols from water.

Since fulvic acids (HFulv) are fairly strong acids (16), with average acid dissociation constants of about 4×10^{-3} and 2×10^{-5}, a low pH is required to force them into the molecular form (eqn. 3). Thus

$$\text{HFulv} \overset{K_a}{\rightleftharpoons} H^+ + \text{Fulv}^- \qquad (3)$$

if the NaOH-NaCl containing eluate from the anion exchange resin is acidified to pH 1, the fulvic acid is predominantly in the molecular form and is adsorbed on the XAD-2 resin. The fulvic acid is eluted from the resin

by a NaOH solution which ionizes it shifting eqn. 3 to the right.

In agreement with the results found by other workers the ultraviolet-visible spectra of our humic materials is featureless (6, 17). However, we did measure some characteristic properties of dilute solutions of humic materials in aqueous 0.1M NaOH. The absortivity values of various humic materials at 465 nm shown in TABLE 4 range from 3.2×10^{-3} $ppm^{-1}cm^{-1}$ for Oyster River fulvic acid to $7.4 \times 10^{-3} ppm^{-1}cm^{-1}$ for Fluka humic acid. Orlov (18) obtained absortivity values between 4×10^{-3} and $11 \times 10^{-3} ppm^{-1}cm^{-1}$ for humic acids extracted from a variety of soils.

The most important spectrophotometric property is the E_4/E_6 ratio, the absorbance ratio at 465 nm and 665 nm. The E_4/E_6 ratios in TABLE 4 range from a high of 10 for Oyster River fulvic acid to a low of 5.2 for the purchased Fluka humic acid. The E_4/E_6 ratios distinguish humic acids from fulvic acids. According to several authors (6, 17, 19), the E_4/E_6 ratio is usually in the 2 to 5 and 6 to 10 range for humic and fulvic acids, respectively. The values of 8.7 for the soil fulvic acid and 5.2 for the Fluka humic acid are typical values. However, the E_4/E_6 ratios for the Oyster River samples are on the high side of the fulvic and humic acid ranges.

Radiocarbon dating studies (20) and a variety of other studies (6, 17) suggest that the E_4/E_6 ratio is directly proportional to the extent of decomposition of humic substances. That is, the samples with high E_4/E_6 values exhibit a low degree of condensation of aromatic portions of humic materials, and are a more recent product of the degredation process. Thus, fulvic acids are expected to have higher E_4/E_6 ratios than humic acids. Our results discussed above are in agreement. The fact that the Oyster River fulvic and humic acids exhibit higher E_4/E_6 ratios than typical soil fulvic

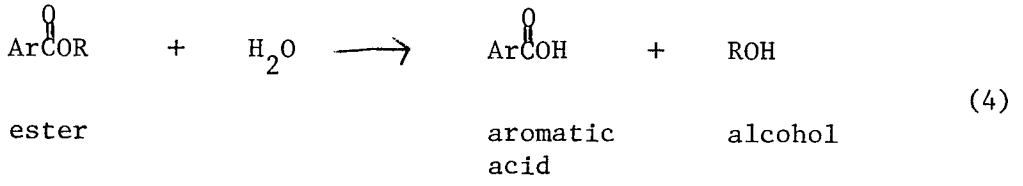
and humic acids might indicate a different degradation process of humic acid in water than in soils.

TABLE 1 lists the elemental analyses, percent ash and pH of a variety of fulvic and humic acid samples. These values are within the usual range expected for humic substances (6).

TABLE 2 compares the organic oxygen-containing functional group analyses for fulvic and humic acid samples that we isolated from a variety of water and soil sources. These values are the first published for water humic and fulvic acid samples, although others (3) have published functional group analyses on total organic matter isolated from water.

The total acidity and carboxyl values are higher in fulvic acid than in humic acid samples irrespective of their source. For example, the Oyster River fulvic acid sample has total acidity and carboxyl values of 10.6 and 6.8 meq g^{-1} respectively, but the Oyster River humic acid sample has lower total acidity (8.2 meq g^{-1}) and carboxyl (4.5 meq g^{-1}) values. A similar relationship occurs between the soil humic acid and fulvic acid samples. In contrast, the phenol OH values are similar for the fulvic acid and the humic acid from a particular soil sample or water sample.

A possible explanation for the higher total acidity and carboxyl values for fulvic acid is based on the known lower average molecular weight of fulvic acid samples (6). The total acidity and carboxyl values, but not the phenol OH values, would increase as humic acid esters hydrolyze. In the hydrolysis reaction the non-acidic ester group $-\overset{O}{\overset{\|}{C}}OR$ breaks down into an aromatic acid and an alchol containing the $-\overset{O}{\overset{\|}{C}}OH$ and -OH groups, respectively (eqn. 4). In agreement the alcohol OH values are generally higher in fulvic



(4)

acid than humic acid (6). Reaction 4 in which humic acid is hydrolyzed into lower molecular weight fulvic acid can occur with water in the soil or in natural water. These conclusions generally agree with the studies of Ogner and Schnitzer (21) and Khan and Schnitzer (22), in which they identified compounds comprising 2% of a fulvic acid sample from soil. They found 28% phenolic acids, 19% benzene carboxylic acids, and other compounds. More recently Neyroud and Schnitzer (23) have shown that similar products occur by oxidation or hydrolysis of humic materials. Thus, the above hydrolysis hypothesis is reasonable.

TABLE 9 puts our functional group analyses in the context of earlier work on soils and on water (3). The table shows the similarity of a variety of fulvic acids to each other and of humic acids to each other. This data suggest the similarity of water to soil humic materials. However, the average Satilla River total acidity and carboxyl values are significantly higher than the Oyster River fulvic acid values. A possible explanation is that the Satilla River samples contained significant amounts of amino acids, which were excluded by our isolation technique.

Number-Average Dissociation-Corrected Molecular Weights $\overline{Mn(corr)}$

The many compounds that comprise soil-derived fulvic acid have been separated into weight fractions by gel filtration and ultramembrane filtration. For example, gel filtration techniques were utilized by Rashid and King (24, 25), Rashid (26), and Kemp and Wong (27) to separate weight fractions of humic materials from lake or ocean sediments. Posner and co-workers (28, 29) have done gel filtration experiments with humic materials isolated from soil. Gjessing (5, 30, 31) has fractionated aquatic humic

materials by ultramembrane filtration. Neither gel filtration nor ultramembrane filtration yield absolute molecular weights for fulvic acids, because of the lack of appropriate calibration materials (6, 27, 29).

In contrast to the relative molecular weights measured by the above methods, absolute $\overline{Mn(corr)}$ values of soil fulvic acid samples have been measured by the colligative property techniques of vapor phase osmometry (14) and of cryoscopy (32). There is no previous $\overline{Mn(corr)}$ data for water fulvic acid.

The preceeding discussion in the Molecular Weight Calculations section on the determination of $\overline{Mn}$ for polymers is for non-electrolytes because it omits any consideration of the dissociation of the solutes. The observed Θ values must be corrected for dissociation of fulvic acid before one can obtain the values of $\underline{a}$ and $\overline{Mn(corr)}$. The dissociation correction has caused considerable difficulty during previous determinations of $\overline{Mn(corr)}$ of all fulvic acid samples.

Hansen and Schnitzer (14) and DeBorger and DeBacker (32) have previously determined $\overline{Mn(corr)}$ values for soil fulvic acid samples. The former researchers obtained a $\overline{Mn(corr)}$ value of 951 for their sample; the latter scientists obtained comparable values of 923, 999, 980 (average value, 967) for three different soil fulvic acid samples. The difficulty with both calculations is inherent in the method of correcting Θ for the dissociation of fulvic acid. Both groups implicitly or explicitly assume that the dissociation of a polybasic acid (H_nA) can be described by a one-step process (eqn. 5) in which C_a and α are the total acid concentration and $[A^{n-}]/C_a$,

$$\begin{array}{ccccc} H_nA & \rightleftharpoons & nH^+ & + & A^{n-} \\ C_a(1-\alpha) & & C_a n\alpha & & C_a\alpha \end{array} \qquad (5)$$

respectively. Based on this assumption, they determined that the total number of particles per mole of H_nA at equilibrium is $1 + n\alpha$ rather than the correct $1 + \alpha_1 + 2\alpha_2 + 3\alpha_3 + \ldots n\alpha_n$. The latter relationship is derived by considering n step-wise equilibria and utilizing the charge balance relationship. As shown below, the correct estimations of the total number of particles per mole of acid is a key to the determination of $\overline{Mn(corr)}$ for fulvic acid.

Results for Model Compounds.

For a dibasic acid H_2A the correction for dissociation can be made on the basis of two dissociation steps (eqn. 6). K_1 and K_2 are the two

$$H_2A \overset{K_1}{\rightleftharpoons} H^+ + HA^- \qquad (6a)$$

$$HA^- \overset{K_2}{\rightleftharpoons} H^+ + A^{2-} \qquad (6b)$$

dissociation equilibrium constants. The total acid concentration can be calculated from a measurement of $[H^+]$ (eqn. 7) according to Freiser and Fernando (33).

$$[H^+]^3 + K_1 [H^+]^2 + (K_1K_2 - K_1C_a) [H^+] - 2K_1K_2C_a = 0 \qquad (7)$$

The only approximation in this equation is that $[H^+] >> [OH^-]$.

For any acid the total concentration of particles at equilibrium is $C_a + [H^+]$. This fact is exemplified for a dibasic acid in eqn. 8. The

$$[H_2A] = [HA^-] = [A^{2-}] = [H^+] = C_a + [H^+] \qquad (8)$$

total number of particles per mole of acid is $1 + [H^+]/C_a$ or $1 + \alpha_1 + 2\alpha_2$. Since the $[H^+]$ is measured and C_a can be calculated from eqn. 7, Θ corrected for dissociation ($\Theta(corr)$) can be determined (eqn. 9). The $\Theta(corr)$

$$\Theta(corr) = \frac{\Theta}{1 + [H^+]/C_a} \qquad (9)$$

values are incorporated into the previously discussed calculations of $\overline{Mn(corr)}$*.

To test our theory we utilized a variety of polycarboxylic acids as well as dextrose and ethyleneglycol. The acids were chosen because of their similarity to polycarboxylic fulvic acids. The results shown in TABLE 6 are (experimental $\overline{Mn(corr)}$, actual molecular weight): dextrose (180, 180) ethyleneglycol (64.9, 62.1), ascorbic acid (181, 177), succinic acid (118, 118), tartaric acid (152, 150), oxalic acid (94.0, 90.1), and trimellitic acid (202, 210). The 2.4% average deviation of the differences between the actual and experimental values indicates that the theory discussed above yields the correct answers. As additional proof we used Hansen and Schnitzer's (14) benzenepentacarboxylic acid data to calculate a $\overline{Mn(corr)}$ value of 300 (theoretical value, 298). Our result compares favorably with their experimental molecular weight of 299. The experimental data for the $\overline{Mn(corr)}$ calculations are in TABLE 7.

Results for Fulvic Acid. Two excellent papers by Gamble (10, 16) have elucidated the dissociation behavior of the same soil fulvic acid sample extensively studied by M. Schnitzer and co-workers (6). Several

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*Although molecular weight, not number-average molecular weight, is the proper term for a pure compound, the latter term will be utilized to simplify the discussion.

results from Gamble's work are important for this paper. (a) Among the mixture of fulvic acids are two groups of acids that are important at the pH of this study. The strongest Type I acids have carboxyl groups adjacent to the phenol OH group; these are the chelating groups. Type II acids include the other carboxylic acids. (b) When the titrations are carried out in the presence of a background electrolyte, 3.2×10^{-3} equiv g^{-1} of Type I groups and 3.4×10^{-3} equiv g^{-1} of Type II groups are found. (c) The average dissociation constants for the Type I and Type II acids, $\overline{K}I$ and $\overline{K}_{II}$, increase with decreasing pH. At the lowest pH values studied $\overline{K}_I$ is 4.7×10^{-3} (pH 2.66) and $\overline{K}_{II}$ is 3.2×10^{-5} (pH 3.57).

Based on Gamble's theory (10, 16) we approximated fulvic acid as a mixture of Type I and Type II monobasic acids, because a polycarboxylic acid model is inappropriate. We utilized Gamble's data to estimate $\overline{K}_I$ and $\overline{K}_{II}$ values of 8.9×10^{-3} and 1.00×10^{-4}, respectively, for our experimental pH range of 1.6 to 2.0. This approximation is justifiable on the basis of previously reported similarities of soil- and water-derived samples of fulvic acid (7).

We determined the amounts of Type I and Type II acids from titrations (9). For water fulvic acid the values for Type I acids (3.0×10^{-3} equiv g^{-1}) and Type II acids (2.9×10^{-3}equiv g^{-1}) are nearly identical. The Type I and Type II values for soil fulvic acid are 3.7×10^{-3} equiv g^{-1} and 3.0×10^{-3} equiv g^{-1}, respectively (TABLE 3).

The equilibria for a mixture of two monobasic acids are expressed by eqn. 10. The $[H^+]$ for the mixture of HA_I and HA_{II} can be expressed (33)

$$HA_I \rightleftharpoons^{K_I} H^+ + A_I^- \qquad (10a)$$

$$HA_{II} \rightleftharpoons^{K_{II}} H^+ + A_{II}^- \qquad (10b)$$

by eqn. 11 in which C_I and C_{II} represent the initial concentration of

$$[H^+] = \frac{K_I C_I}{[H^+] + K_I} + \frac{K_{II} C_{II}}{[H^+] + K_{II}} \qquad (11)$$

Type I and Type II acids. Since the ratio of equivalents per gram of Type I and II acids is known for both fulvic acid samples (e.g. for water fulvic acid, $C_I/C_{II} = 1.03$), both C_I and C_{II} can be calculated via eqn. 11.

The total number of particles for a mixture of two monobasic acids can be easily expressed (eqn. 12). The total number of particles per total

$$[HA_I] + [HA_{II}] + [A_I^-] + [A_{II}^-] + [H^+] = C_I + C_{II} + [H^+] \qquad (12)$$

moles of acid $(C_I + C_{II})$ is $1 + [H^+]/(C_I + C_{II})$ or $1 + (C_I\alpha_I + C_{II}\alpha_{II})/(C_I + C_{II})$. As previously discussed for the dibasic acid case, the measured Θ can be corrected for dissociation (eqn. 13).

$$\Theta(corr) = \frac{\Theta}{1 + [H^+]/(C_I + C_{II})} \qquad (13)$$

We tested the calculations of eqn. 11 and 13 with a mixture of tartaric acid (HA_I) and succinic acid (HA_{II}) in which $C_I/C_{II} = 1.55$. Despite ignoring the second dissociation steps of these dibasic acids the actual molecular weight of 138 compared very favorably to our 142 observed value.

TABLE 7 includes experimental data for tartaric acid, the model compounds, a succinic acid-tartaric acid mixture, and soil and water fulvic acids. We utilized the zero point method (Appendix I) to calculate a (eqn. 1),

and the a is used to calculate $\overline{Mn(corr)}$ (eqn. 2). Our approach is justified by the previously mentioned small errors in the accuracy of $\overline{Mn(corr)}$ for the other model compounds and the mixture, and by the soundness of the theoretical approach. Thus, we have confidence in our $\overline{Mn(Corr)}$ values of 644 and 626 for the fulvic acids isolated from soil and water, respectively.

We also recalculated the fulvic acid $\overline{Mn(corr)}$ from Hansen and Schnitzer's data (14) using our method of analysis and the data of Gamble (10, 16). The recalculated value of 615 for soil fulvic acid compares favorably with our 644 soil fulvic acid value (TABLE 7).

Electron Spin Resonance Experiments

Steelink et al. (34-36) and Riffaldi and Schnitzer (37) have made important contributions to the interpretation of the esr spectra of humic acids and their sodium salts. The latter paper and the book by Schnitzer and Khan (6) review the literature of esr studies of humic materials. Their results, which were primarily of humic acids in the solid state, lead to several conclusions. (a) Class I humic acids have four peaks and class II humic acids have one featureless peak. (b) The g-values are between 2.0030 and 2.0040 in most cases. (c) The line widths range from about 1.8 to 6.5 gauss. (d) The spin concentration is much lower for the humic acids than for their sodium salts. (e) The signal intensity is greater for humic acids than fulvic acids.

The research discussed above omits many important aspects of the esr and redox behavior of the humic materials which we will discuss here. In particular this section of the report includes discussions of (a) the solid state and aqueous solution pH-dependent esr spectra of aquatic and soil fulvic and humic acids, (b) the relationships between the aqueous esr signal intensities and reduction potentials (E_h), and (c) the proof of existence and

quantitative determination of semiquinone radicals in humic materials.

TABLE 10 shows the results of our solid state esr studies on aquatic and soil fulvic and humic acids. The esr spectra are of the class II type with one band that is asymmetric. The g-values of 2.0037 and 2.0038 are near the high edge of the usual humic acid range of 2.0030 to 2.0040. In accord with previous work (6, 37) the line widths of the aquatic and soil samples vary from 3.3 gauss to 5.6 guass. The results in TABLE 10 also agree with earlier solid state results on soil humic materials in that the spin concentration of humic acid is greater than that for fulvic acid (37). The threefold spin concentration increase occurs for both the aquatic and the soil samples. The two aquatic samples have about 50% of the spin concentration of the corresponding soil fulvic and humic acid samples. Thus the order of decreasing spin concentration is: soil humic acid > aquatic humic acid > soil fulvic acid > aquatic fulvic acid.

Data of this nature were used in the past to suggest that the free radicals are caused by semiquinone compounds. (6, 35, 37). This conclusion is reached despite the fact that the observed humic acid g-values are lower than the 2.0051 ± 0.0007 values observed for model semiquinones like ortho- and para- benzosemiquinones (38). However, the following aqueous solution esr studies prove conclusively that the free radical content in humic materials is exclusively or predominantly due to semiquinones.

As shown in TABLE 11 there is a general increase in spin concentration (I) for both fulvic acid samples as the pH is increased. Closer inspection of TABLE 11 reveals that I is essentially constant between pH 2 and 7, but it increases sharply above pH 9. The combined data of TABLE 11 gives a linear graph when $\log((I-I_A)/(I_B-I))$ is plotted against pH. I, I_A, and I_B are spin concentrations at intermediate pH, low pH (acid form), and high

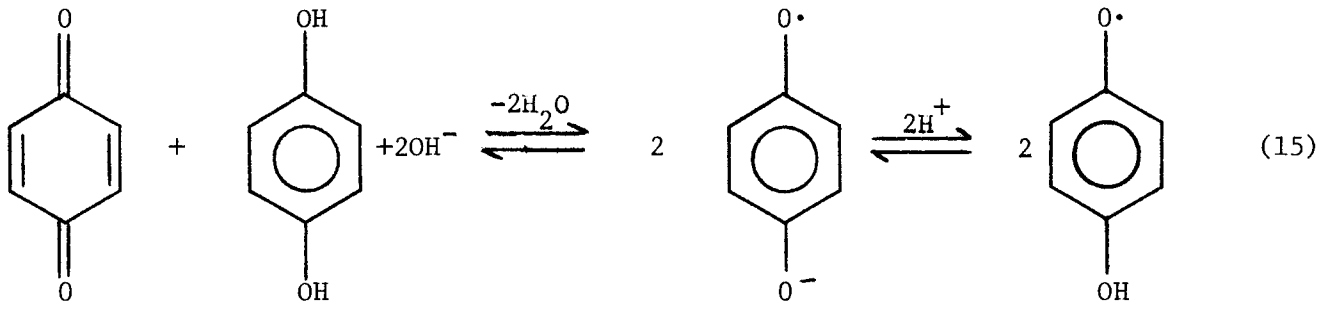
pH (base form). A plot of the data in TABLE 11 shows an intercept of 10.1 and a slope of 1.8 (correlation coefficient, 0.94).

This behavior is explainable by recognizing that the graph is a representation of the Henderson-Hasselbalch equation in which $I-I_A$ and $I-I_B$ are, respectively, proportional to the concentrations of the base and the acid from the species (eqn. 14). The term $\overline{pK_a}$ in eqn. 14 is the average

$$pH = \overline{pK_a} + \frac{1}{n} \log \frac{I-I_A}{I_B-I} \qquad (14)$$

of the pK_a values of a polyprotic acid and $\underline{n}$ is the number of equivalents of H^+ per mole of the acid. Thus, the slope reveals that the free radical precursor is not a single pure acid ($\underline{n}$=0.55), and the intercept reveals that the acids have an average pK_a value of 10.1.

These aqueous solution esr results can be explained by considering the various pH dependent equilibria of the model compound $\underline{p}$-benzosemiquinone (eqn. 15). Although we will explain the data based on eqn. 15, it should be



(15)

quinone(Q) hydroquinone (H_2Q) semiquinone anion ($Q\cdot^-$) semiquinone ($HQ\cdot$)

emphasized that fulvic acid contains a wide variety of semiquinones and related compounds (7). This fact augmented by Gamble's (10, 16) results explains why $\underline{n}$ is not a whole number.

The residual esr signal between pH 2 and 7 and that in the solid state can be explained by the presence of $HQ^{\bullet}$, which has a pK_a value (38) of 4, or by a pH independent radical. However, the low pH radical is only a small fraction of the free radical content of a strongly basic aqueous solution. The enhanced signal at high pH shown in TABLE 11 is primarily due to the reaction of Q and H_2Q to form $Q^{\bullet}$ as shown in eqn. 15. Since the pK values of H_2Q (39) are 9.85 and 11.4, the intercept of 10.1 from the data in TABLE 11 represents a reasonable $\overline{pK_a}$ value. The reason that little increase in spin concentration occurs below pH 7 is that the phenol protons are negligibly dissociated (eqn. 15). In agreement with this result other workers found that the esr spin concentration increased by a factor of 15 between humic acid and its sodium salt (35). The disappearance of the esr signal at high pH upon the introduction of oxygen is due to the well-known oxidation of $Q^{\bullet -}$ to Q (38).

The reduction potentials (E_h) of the fulvic acid samples shown in TABLE 8 agree with the above analysis, because between pH 2.9 and 6.9 the E_h values are inversely proportional to the pH. The highest E_h value of about 0.50 volts occurs when the E_h-pH data of both fulvic acid samples are extrapolated to pH 0. This trend means that H^+ is released when fulvic acid acts as a reducing agent and that it is a stronger reducing agent in basic solution. This result agrees with our electrolysis studies at pH 11.2. We found that a potential of +0.20 volts (vs. SCE) for 25 min decreases the esr signal by 19%, and that an additional 60 min at 0.40 volts cause a further 6% signal reduction. Others have observed similar behavior as humic acid reduced Hg^{2+} to Hg° (40). All these results demonstrate that semiquinones are partially or wholly responsible for the reducing power of humic materials (eqn. 16).

$$H^+ + Q\cdot^- \underset{pK_a \simeq 4}{\rightleftharpoons} HQ\cdot \longrightarrow H^+ + Q + e \qquad (16)$$

Because of the demonstration that the free radical content of humic materials is caused predominantly or entirely by the semiquinone radicals (HQ· and $Q\cdot^-$), the concentration of the radical can be quantified. We did this by use of a Fremy's salt standard solution. The aquatic fulvic acid, for example, has an average of 3.5×10^{17} spins g^{-1} or 3.5×10^{17} semiquinone molecules g^{-1} from pH 2.4 to 7. The values are proportional to the signal area at higher pH values in TABLE 11.

TABLE 12 shows the relationships among the pH, absorbance and spin concentration of aqueous solutions of Podzol soil fulvic acid. Contrary to previous solid state results (6, 37), the absorbance at 465 nm is not directly proportional to the spin concentrations of the solutions. Between pH 2.42 and 12.10 the spin concentration increases by a factor of 17, but the absorbance does not even double.

CONCLUSIONS

The research described in this report and our unpublished research without exception indicate that fulvic acids from water and soil (and humic acids from water and soil) are similar. This is important from an experimental point of view because infinitely more is known about soil organic matter than water organic matter. Thus we can utilize the easily obtained soil organic matter as model compounds in initial tests, before we use the hard-won water organic matter. An additional advantage of the similarity of the water- and soil-originated materials is that we can utilize the brilliant research of Schnitzer and co-workers and of others reviewed in the book by Schnitzer and Khan (6). Their results are guides to pertinent, future research on water fulvic and humic acids. This synergic approach will probably be the most effective way to elucidate the nature of water organic matter.

An important aspect of this research is the breaking down of the artificial barrier of humic materials research between soil and water scientists. Because of the complexity of many experimental techniques, it is often difficult to correlate the results of different research groups. For this reason we always do side-by-side studies of water and soil humic materials.

Because the carboxyl, phenol OH, and carbonyl groups are all good electron donors to metal ions, it is apparent that there is significant organic matter-metal ion interaction in lakes and rivers. Research in progress is aimed at studying the nature of metal complexes formed between fulvic acid and metal ions found in rivers and lakes.

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APPENDIX I. MOLECULAR WEIGHT CALCULATIONS

Excellent overviews of the nature of the molecular weight calculations of polymers are presented in two books (11, 12). Two basic equations are used to calculate $\overline{Mn}$ values. They relate the instrument readout Θ to the W (grams of solute per kilogram of solvent)(eqn. 17) and the constants $\underline{a}$

$$\Theta = \underline{a}\, W + \underline{b}\, W^2 + \cdots \tag{17}$$

and K_{app} (the apparatus constant) to the $\overline{Mn}$ value (eqn. 18).

$$\overline{Mn} = K_{app}/a \tag{18}$$

In the zero point point calculation the first set of data points (Θ_1, W_1) are subtracted from each of the other data points (Θ_i, W_i). This is done to negate any potential zero point error, that is a non-zero Θ when W=0. We used $\delta=\Theta_i-\Theta_1$ and $Z=W_i-W_1$ in eqn. 19 and 20 to calculate

$$\underline{a}' = \frac{(\Sigma Z\delta)(\Sigma Z^4) - (\Sigma Z^2\delta)(\Sigma Z^3)}{(\Sigma Z^2)(\Sigma Z^4) - (\Sigma Z^3)(\Sigma Z^3)} \tag{19}$$

$$\underline{b} = \frac{(\Sigma Z^2\Delta)(\Sigma Z^2) - (\Sigma Z\delta)(\Sigma Z^3)}{(\Sigma Z^2)(\Sigma Z^4) - (\Sigma Z^3)(\Sigma Z^3)} \tag{20}$$

$\underline{a}^1$ and $\underline{b}$. The symbol $\underline{b}$ is the same as in eqn. 17, and $\underline{a}^1$ is related to $\underline{a}$ of eqn. 17 and 18 by $\underline{a} = \underline{a}^1 - 2\underline{b}W_1$. Then we utilize $\underline{a}$ and K_{app} in eqn. 18 to calculate $\overline{Mn}$. To calculate $\overline{Mn(corr)}$ for the acids we utilize Θ(corr) instead of Θ. The entire calculation is done by a computer program. A variety of other computational approaches are given in references 11 and 12.

APPENDIX II. ANALYSIS OF ELECTRON SPIN RESONANCE SIGNAL AREAS BY THE FIRST MOMENT METHOD

Since esr readout curves are first derivative plots, it is necessary to perform a double integration to calculate their area. The double integration can be approximated by Newton's method or the first moment method which we used.

In the first moment approach the esr curve is broken up into intervals (x) of approximately 1 or 2 mm. Start at the point where the esr curve changes sign and measure the absolute value of the height (h_i). Then x and h_i are multiplied according to the following scheme.

$$(x)(h_1)=y_1$$

$$(2x)(h_2)=y_2$$

$$(3x)(h_3)=y_3$$

The area of the absorption curve, which is proportional to the spin concentration is $\sum_{i=1}^{n} y_i$ where n is the total number of x intervals.

TABLE 1. Elemental Analysis, Percent Ash, and pH of Aquatic and Soil Fulvic and Humic Acids[a]

	% anal.				
Sample	C	H	N	%Ash	pH[b]
S-FA(C)	53.1	3.24	0.90	0.8	1.80
OR-FA	51.1	3.62	1.13	1.0	2.08
JP(A)-FA	45.7	4.26	1.57	7.1[d]	2.18
JP(B)-FA	41.6	4.17	1.00	3.8[d]	2.40
S-HA(C)[C]	53.8	3.88	2.45	1.6	
OR-HA	53.4	3.73	2.10	4.3[d]	-
JP-HA	59.5	5.11	1.95	1.8	-
Fluka HA[c]	57.7	4.54	0.83	1.9	-

[a]Abb: S, soil; FA, fulvic acid; C, Conway, N.H.; HA, humic acid; OR, Oyster River; JP(A), Jewell Pond (acetone treatment); JP(B), Jewell Pond (butanol treatment). These treatments are discussed in reference 7.

[b]Concentration is 10 mg ml^{-1}.

[c]Purified, including HCl-HF treatment (see EXPERIMENTAL AND CALCULATIONS SECTION).

[d]Insufficient sample to warrent further purification.

TABLE 2. Organic Functional Group Analysis of Aquatic and Soil Fulvic and Humic Acids (meq g^{-1})[a]

Sample	Total Acidity	Carboxyl	Phenol OH[b]	Carbonyl
S-FA(C)	13.4	8.2	5.2	3.5
OR-FA	10.6	6.8	4.3	4.3
JP(A)-FA	9.6	8.1	1.5	6.2
JP(B)-FA	10.5	7.6	2.9	7.4
S-HA(C)[d]	8.1	4.5	3.6	3.0
OR-HA	8.2	4.5	3.7	4.3
JP-HA	7.1	4.9	2.2	5.1
Fluka HA[c]	7.1	4.2	2.9	5.7

[a]Abb: meq g^{-1}, milliequivalents per gram. See TABLE 1 for other abbreviations.

[b]Difference between total acidity and carboxyl values.

[c]Purified (see EXPERIMENTAL AND CALCULATIONS section).

[d]Purified, including HCl-HF treatment (see EXPERIMENTAL AND CALCULATIONS section).

TABLE 3. Concentrations of Aquatic and Soil Fulvic Acid (FA) Chelating Groups and Carboxyl Groups

Sample	Chelating Groups,[a] meq g^{-1}	Carboxyl Groups, meq g^{-1}
Oyster River FA	3.0	5.9
Podzol Soil FA	3.7	6.7

[a]Carboxyl groups ortho to phenol OH groups.

TABLE 4. Spectroscopic Properties of Aquatic and Soil Fulvic and Humic Acids[a]

Sample	Absorbance 465 nm	Absorbance 665 nm	E_4/E_6	Conc. (ppm)	Absorptivity x10^3 (ppm^{-1} cm^{-1})[b]
OR-FA	0.30	0.030	10	95	3.2
S-FA(C)	0.35	0.040	8.7	103	3.4
OR-HA	0.49	0.080	6.1	143	3.4
S-HA(C)[c]	0.45	0.067	6.7	97	4.7
Fluka HA[c]	0.86	0.169	5.2	116	7.4

[a]See TABLE 1 for abbreviations.

[b]At 465 nm.

[c]Purified, including HCl-HF treatment (see EXPERIMENTAL AND CALCULATIONS section).

TABLE 5. The Absorbance at 665 nm of Aqueous Podzol Fulvic Acid Solutions[a] as a Function of pH

pH	Absorbance
1.60	0.330
3.45	0.370
4.32	0.370
5.09	0.385
6.30	0.420
7.10	0.452
9.00	0.542
10.15	0.594
11.88	0.619

[a]concentration is 228 ppm.

TABLE 6. Theoretical and Experimental $\overline{Mn(corr)}$ Values of Model Compounds

Compound	Theoretical Molecular Weight	Experimental Mn(corr)	% Deviation from Theoretical value
Dextrose	180	180	0.0
Ethyleneglycol	62.1	64.9	+4.5
L-Ascorbic Acid	177	181	+2.2
Succinic Acid	118	118	0.0
Tartaric Acid	150	152	+2.0
Oxalic Acid	90.1	94.0	+3.3
Trimellitic Acid	211	201	-4.1
Benzenepenta-carboxylic Acid[a]	298	300[a]	+0.7
Tartaric-Succinic Acid Mixture	138	142	+2.9

[a]Calculated from data of reference 14.

TABLE 7. Sample Data for Calculations of $\overline{Mn(corr)}$

Compound	W, g/Kg H_2O	pH	Θ	Θ(corr)	a	$\overline{Mn(corr)}$
Dextrose	18.90		109.0			
	21.46		123.0			
	40.17		227.4			
	43.23		243.2			
	64.75		369.8			
	74.84		424.8			
	80.89		455.8			
					5.56	180
Ethyleneglycol	7.37		121.2			
	12.83		208.7			
	16.33		263.0			
	16.56		265.6			
	24.03		386.6			
	25.58		413.4			
	25.84		415.3			
					15.47	64.9
L-Ascorbic Acid	5.51	2.78	32.8	31.6		
	14.61	2.63	84.3	82.0		
	19.94	2.58	113.1	110.4		
	24.65	2.53	139.4	136.3		
	42.86	2.40	238.0	243.1		
					5.55	181
Succinic Acid	3.23	2.88	29.1	27.9		
	5.44	2.72	47.6	46.2		
	6.82	2.64	58.8	57.3		
	8.49	2.61	73.5	71.8		
	9.49	2.59	81.0	79.1		
	13.90	2.54	117.2	114.8		
					8.51	118
Tartaric Acid	2.00	2.47	17.2	14.2		
	4.00	2.32	31.8	27.4		
	6.00	2.22	45.9	40.5		
	8.00	2.16	59.9	53.7		
	10.00	2.11	73.9	66.9		
	12.00	2.07	87.7	80.0		
					6.59	152

TABLE 7. Continued

Compound	W, g/Kg H_2O	pH	Θ	Θ(corr)	a	$\overline{Mn(corr)}$
Oxalic Acid	1.70	1.92	34.1	18.7		
	3.24	1.71	60.1	34.4		
	7.74	1.46	130.4	80.5		
	9.40	1.41	155.3	97.8		
	11.27	1.36	181.8	116.5		
	13.01	1.32	207.6	134.8		
					10.68	94.0
Trimellitic Acid	4.42	2.18	31.0	23.4		
	5.65	2.14	37.0	28.4		
	7.18	2.10	43.9	34.2		
	9.10	2.06	51.9	41.0		
					5.00	201
Benzenepenta-carboxylic Acid	8.00	1.83	3.02	1.95		
	10.00	1.76	3.72	2.47		
	12.00	1.70	4.42	3.01		
	15.00	1.64	5.46	3.82		
	17.00	1.60	6.16	4.38		
	20.00	1.55	7.22	5.24		
					0.236	300[a]
Tartaric-Succinic Acid Mixture	2.00	2.59	16.5	14.1		
	4.00	2.44	31.8	28.2		
	6.00	2.36	46.9	42.3		
	8.00	2.29	61.8	56.4		
	10.00	2.24	76.7	70.5		
	12.00	2.21	91.4	84.6		
					7.05	142
Soil Fulvic Acid	2.00	2.52	6.1	4.3		
	4.00	2.31	10.1	7.4		
	6.00	2.19	14.0	10.5		
	8.00	2.10	17.8	13.7		
	10.00	2.03	21.5	16.8		
	12.00	1.98	25.1	19.9		
					1.561	643[b]

TABLE 7. Continued

Compound	W, g/Kg H_2O	pH	Θ	Θ(corr)	a	$\overline{Mn(corr)}$
Aquatic Fulvic Acid	2.00	2.63	1.8	1.3		
	4.00	2.36	6.1	4.5		
	6.00	2.21	9.9	7.6		
	8.00	2.10	13.8	10.8		
	10.00	2.02	17.4	13.9		
	12.00	1.95	21.0	17.1		
					1.586	633[b]
Soil Fulvic Acid[a]	8.00	2.085	1.205	0.958		
	10.00	2.006	1.480	1.119		
	12.00	1.945	1.755	1.442		
	15.00	1.870	2.167	1.812		
	17.00	1.830	2.442	2.061		
	20.00	1.772	2.854	2.440		
					0.1148	616

[a] W, pH, and Θ data from reference 14; Θ(corr) calculated as shown in this paper. K_{app} is 70.75 kg ohmg^{-1}.

[b] The values are different from those discussed in the text, because they are an average of replicate measurements.

TABLE 8. Reduction Potentials (E_h) of Aquatic and Soil Fulvic Acids (FA)

Oyster River FA		Podzol Soil FA	
pH	E_h, Volts	pH	E_h, Volts
0.00[a]	0.49[a]	0.00[a]	0.51[a]
2.89	0.40	2.92	0.39
4.20	0.34	3.84	0.34
4.89	0.32	5.02	0.29
5.64	0.30	5.71	0.28
6.76	0.26	6.90	0.25

[a]Extrapolated value (see Measurement of Reduction Potentials (E_h) of Fulvic Acids section).

TABLE 9. Oxygen-Containing Functional Groups of Aquatic and Soil Fulvic and Humic Acids (meq g^{-1})[a]

Sample	Total Acidity	Carboxyl	Phenol OH[b]	Carbonyl
S-HA[c]	7.9	3.7	4.2	3.1
S-HA(C)[d,f]	8.1	4.5	3.6	3.0
OR-HA[d]	8.2	4.5	3.7	4.3
JP-HA[d]	7.1	4.9	2.2	5.1
S-FA[c]	12.8	8.9	3.9	2.0
S-FA(C)[d]	13.4	8.2	5.2	3.5
OR-FA[d]	10.6	6.8	4.3	4.3
SR-OM[e]	13.7	8.8	5.0	-

[a]Abb: meq g^{-1}, milliequivalents per gram; SR, Satilla River; OM, organic matter. See TABLE 1 for other abbreviations.

[b]Difference between total acidity and carboxyl values.

[c]Average values (reference 6, Chap. 3).

[d]This work.

[e]Average value for all organic matter from waters of the Satilla River system in southeast Georgia (3).

[f]Purified, including HCl-HF treatment (see EXPERIMENTAL AND CALCULATIONS section).

TABLE 10. Solid State Electron Spin Resonance Spectra of Aquatic and Soil Fulvic Acids (FA) and Humic Acids (HA)

Sample	g-value[a]	Line Width, Gauss	Relative Spin[a] Concentration
Oyster River FA	2.0038	4.0	1.00
Podzol Soil FA	2.0037	3.3	1.91
Oyster River HA	2.0038	5.6	3.18
Podzol Soil HA	2.0038	4.0	5.87

[a]The error in g-values is $\pm$0.0002 and the relative spin concentration error is $\pm$3%.

TABLE 11. The pH Dependence of Spin Concentration in Aqueous Solutions of Aquatic and Soil Fulvic Acids (FA)[a]

Oyster River FA			Podzol Soil FA		
pH	I, spins $g^{-1}x10^{-17}$	$\log(\frac{I-I_A}{I_B-I})$	pH	I, spins $g^{-1}x10^{-17}$	$\log(\frac{I-I_A}{I_B-I})$
2.40	4.91	-1.026	2.42	2.25	-2.106
4.45	$2.72(I_A)$	-----	4.90	2.06	-2.601
5.90	3.39	-1.544	6.52	$0.94(I_A)$	-----
6.85	2.92	-2.100	9.50	14.5	-0.273
9.50	9.80	-0.412	9.70	20.1	-0.00554
10.26	14.9	-0.0386	10.81	12.4	-0.378
11.92	16.6	+0.0788	11.20	33.6	+0.857
12.35	$28.1(I_B)$	-----	11.70	$37.9(I_B)$	-----
			11.95	32.8	0.773
			12.10	37.4	1.771

[a]abb: I, I_A, and I_B are intermediate, minimum (acid form), and maximum (base form) spin concentrations, respectively.

TABLE 12. The pH, Absorbance, and Spin Concentration Relationships for Aqueous Solutions of Soil Fulvic Acid

pH	Absorbance[a]	I, spins $g^{-1} \times 10^{-17}$
2.42	0.342	2.25
4.90	0.381	2.06
6.52	0.425	0.94
9.50	0.563	14.5
9.70	0.572	20.1
10.81	0.606	12.4
11.20	0.612	33.6
11.70	0.617	37.9
11.95	0.620	32.8
12.10	0.622	37.4

[a]Absorbance at 465 nm with solutions containing 228 ppm fulvic acid. The absorbance-pH data were plotted, and the absorbance values were read off at the pH corresponding to the esr measurements.