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Dissolved Humic Substances: Characterization of their Sorption onto Iron Hydroxide Aluminum Hydroxide Kaolinite and Smectite

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**Dissolved Humic Substances: Characterization of their sorption onto
Iron hydroxide, Aluminum hydroxide, Kaolinite and Smectite**

by

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B.S. May 2000, Coastal Carolina University

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

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ABSTRACT

Dissolved Humic Substances: Characterization of their sorption onto Iron hydroxide, Aluminum Hydroxide, Kaolinite and Smectite

Sarah E. Diebel
Old Dominion University, 2003

Director: Dr. Joseph Rule

Humic (HA) and fulvic (FA) acids participate in many geochemical processes and the mechanisms of their sorption to colloidal surfaces are numerous, indicating these substances are complex and heterogeneous. The current study focuses on the interactions of the Fe hydroxide (FE), Al hydroxide, Kaolinite (KA) and Smectite (SM) and these naturally occurring organic acids using Fourier Transform Infrared Spectroscopy (FTIR) and DOC analysis at pH 4. FTIR was used for the initial functional group characterization, as well as the comparison of before and after adsorption spectra of the HA and FA. DOC examined the amounts of HA and FA adsorbed to the four colloids.

An important finding was the spectral patterns of both FA and HA changed when the sample was characterized from a concentrated versus a diluted solution. Concentrated solutions are usually produced by the most common isolation techniques and samples are subsequently diluted for experimental procedures. These results underscore the importance of conducting FA and HA spectral characterization under exact experimental conditions.

Generally, the peaks associated with carboxyl groups showed a decrease in intensity after exposure to FE and only slightly with AL. The peaks corresponding to phenolic groups decreased after exposure to SM and AL. Contrary to other viewpoints, H-bonding seemed be a major type of adsorption mechanism based on changes in the

recorded spectra. From the DOC analysis two important observations were made, more HA was adsorbed by all four colloids compared to FA and FE adsorbed more HA and FA than the other three colloids.

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This thesis is dedicated to my caring and devoted
husband, Nathan D. Diebel

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CHAPTER I

INTRODUCTION

Humic substances are a common component in natural systems and will inevitably complex with minerals suspended as sediment or from the soil environment (Kubicki et al., 1999). Coatings of humic substances (HS) most certainly alter physicochemical properties of the colloidal minerals including the electrophoretic mobility, sorption characteristics, colloidal stability, transport and interaction with contaminants. Humic substances also interact with inorganic and organic contaminants affecting behaviors such as partitioning, solubility, transport and fate (Gu et al., 1996). Other important influences include the growth and dissolution of minerals, cycling of trace metals and the global C budget (Namjesnik-Dejanovic et al., 2000).

The mechanisms by which humic substances and organic matter are adsorbed onto mineral surfaces are not yet fully understood. However, results show that the adsorption of organic acids by mineral surfaces is dependent on pH and electrolyte concentration (Filius et al., 2000). Gu et al. (1995) studied the pH adsorption of organic acids containing carboxylic and phenolic groups onto iron hydroxides. The compounds with carboxylic groups exhibited an adsorption maximum at low pH, whereas compounds containing phenolic groups show an adsorption maximum at high pH. These results imply that carboxylic groups and phenolic groups are important to the binding mechanisms associated with organic matter at different solution pHs.

Methods used in the characterization of organic matter include HPLC, UV/VIS, fluorescence, and classical spectroscopic methods such as FTIR. These methods of characterization are useful for understanding the behavior of humic substances.

The development of predictive behavior of humic substances in regards to their multifunctional role in the environment is necessary in order to understand other aspects of humic substances, such as their structural and compositional framework.

Dissolved Organic Matter

Aquatic environments including streams and rivers, lakes, wetlands, groundwater and marine systems consist of a complex class of organic molecules known as dissolved organic matter (DOM). Within this complex pool of DOM, which comprises 50 to 75 percent of the dissolved organic carbon (DOC) found in natural waters, is a group of polymeric organic acids of varying sizes and shapes otherwise known as humic substances (HS). Fifty percent of the DOC in surface water of rivers in the United States occurs as humic substances (Fig. 1; Thurman, 1985).

Many sources contribute to the origination of stream humic substances. One portion of stream humic substances, the allochthonous source, is delivered by wind and water from the soil, litter and decomposing land vegetation. This portion is a primary contributor of dissolved organic carbon (DOC) and particulate organic carbon (POC) into streams and small to moderate sized rivers. The other portion, the autochthonous source, results from the decaying of aquatic plants and animal remains and groundwater. Aquatic humic substances have also been shown to originate from sewage effluents (Malcolm, 1985). It is important to note that humic substances found in the soil environment have

similar chemical properties as those found in natural water systems. Namjesnik-Dejanovic et al. (2000) states that natural organic matter (NOM) found in surface water represents material that has passed through soils and sediments. Other studies on the transport of humic substances lead to the conclusion that 50 percent of the oceanic DOC originated in freshwater and terrestrial environments (Aiken et al., 1985). Therefore, humic substances are widely distributed in a variety of environments and are a ubiquitous part of the natural world.

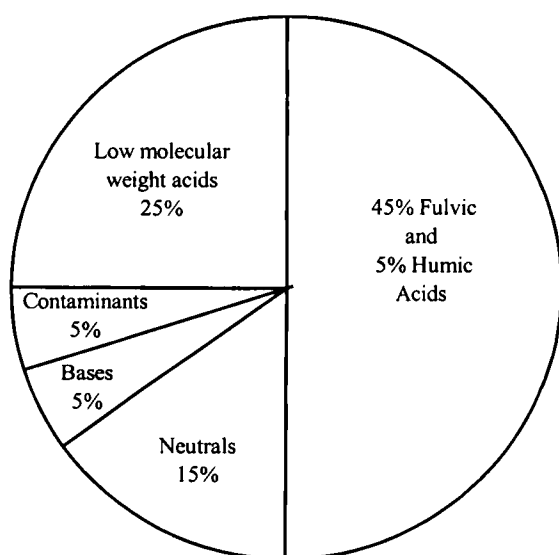


Fig. 1. Distribution of surface water DOC in rivers of the United States (Thurman, 1985).

Humic substances contain a variety of diverse chemical functionalities and there are many different analytical methods used to isolate and fractionate this material. Consequently, they can only be operationally defined based on the method used for

isolation (Peuravuori et al., 2002). The method of isolation (Fig. 2) used in this project classifies humic substances into three chemical divisions based on their solubility in water. They are separated into insoluble humin, alkali (above pH 2) soluble humic acid (HA) and fulvic acid (FA), which is the fraction that is acid and base soluble (Brady and Weil, 2002; McBride, 1994). Isolation of HA and FA is accomplished by precipitating the HA fraction at pH 1. At this pH, HA are less soluble due to fewer carboxylate anions. HA are also higher in molecular weight. Lastly, HA are higher in ash content and phenolic content than FA. These three factors account for differing solubilities of HA and FA, permitting their separation from each other. Approximately 90 percent of the HS found in natural waters occur as fulvic acids, while the other 10 percent or less occur as humic acid. Therefore, fulvic acid exists as a solute in natural waters more commonly than humic acid (Thurman, 1985).

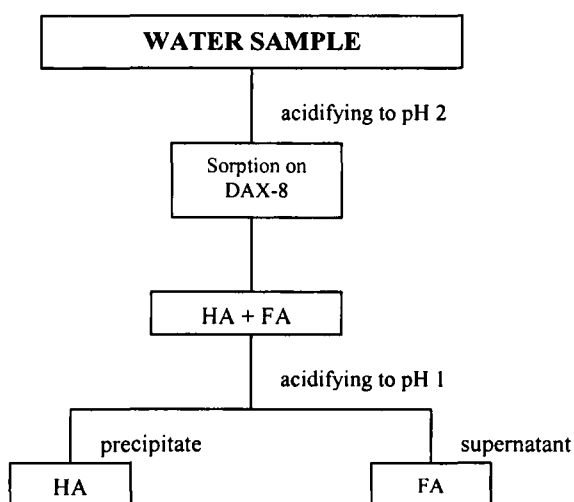


Fig. 2. Isolation of humic substances from aquatic samples (Janoš, 2003).

Elementally, humic substances are composed of approximately 50% Carbon, 35-40% Oxygen, 4-5% Hydrogen, 1-2% Nitrogen and less than 1% Phosphorus and Sulfur (Thurman, 1985). The chemical variability of HS results in differences in their physical characteristics (Aiken et al., 1985). Table 1 lists these differences including molecular weight, color and carbon and oxygen content. The molecular weight of FA varies from 500 to 2,000 g mol⁻¹, HA ranges from 2,000 to 5,000 g mol⁻¹ and humin is greater than 100,000 g mol⁻¹ (Thurman et al., 1982). This property is important because larger molecular weight humic substances are more hydrophobic (less polar), which is an important factor controlling proton and metal binding and organic pollutant partitioning. Studies have found that higher molecular weight and more aromatic and more hydrophobic components may adsorb preferentially to different types of mineral surfaces (Meier et al., 1999).

Table 1. Physical properties of humic substances (Thurman et al., 1982).

	FA	HA	Humin
Molecular Weight	500-2,000	2,000-5,000	>100,000
Color	light yellow-brown	dark brown-gray	black
% Carbon	41-53	54-59	62
% Oxygen	40-50	33-39	30
————— increase in C content —————>			
————— decrease in O content —————>			
————— decrease in exchange acidity —————>			
————— decrease in degree of solubility —————>			

Chemical and physical characteristics of stream humic substances are determined by specific functional groups contained in their structure. These functional groups are

classified based on their acidic, basic or neutral properties, which refer to their ability to accept or donate a proton. Therefore, they interact with water by hydrogen bonding, increasing the solubility of the organic molecule. There are at least twelve functional groups that are important components of NOM. Of the twelve, the carboxyl and phenolic functional groups determine many of the stream humic substance's properties (Thurman, 1985).

The humic material occurring in soils and sediments are bound in various ways, including (1) As insoluble macromolecular complexes, (2) As macromolecular complexes bound together by di- and tri- valent cations such as Ca^{2+} , Fe^{3+} , and Al^{3+} , and (3) In combination with clay minerals, through bridging of polyvalent cations (clay-metal-humus), hydrogen bonding, or van der Waal's forces. Insoluble macromolecular complexes are important in peat and other types of organic-rich sediments, where clay and metal complexes are low in relation to the amount of humus. The binding of di- and tri- valent cations to macromolecular complexes develops under certain climatic and biological conditions and results in the mobilization and transport of iron, aluminum and organic matter (Stevenson, 1985).

Clay minerals are typically coated with a layer of hydrous oxides, which then become strong absorbents of humic substances. Figure 3 depicts the clay-metal-humic complex found in soils and sediments. Fe^{3+} and Al^{3+} are important ions because they form strong bonds with the humic substances and coordination complexes with organic compounds. In contrast, Ca^{2+} does not form strong complexes or bonds because this cation is easily exchanged and displaced (Stevenson, 1985).

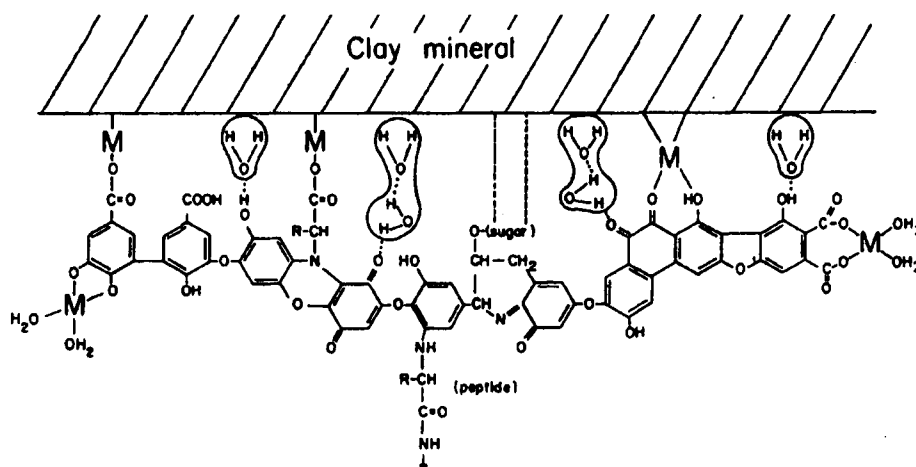


Fig. 3. Schematic diagram of a clay-metal-humic complex (Stevenson, 1985).

Humic Substances—Chemical Properties

The chemical properties of humic substances are a product of the functional groups found within their structure. It is quite apparent that humic substances consist of a heterogeneous mixture of compounds, of which no exact structural formula can be given. The two fractions, humic and fulvic acids, have precisely the same structure or array of functional groups. Important functional groups of aquatic humic substances (Table 2) are classified based on their acidic, basic or neutral properties (Thurman, 1985).

Fulvic acids are lower in molecular weight and consist of higher oxygen, but smaller carbon contents compared to the higher molecular weight humic acid. Mostly all the oxygen found in fulvic acid can be recognized in known functional groups (COOH, OH, C=O). The proportion of oxygen found in humic acids is a structural component of the nucleus (Thurman, 1985).

Table 2. Important functional groups of dissolved organic carbon. R is an aliphatic backbone, and Ar is an aromatic ring (Thurman, 1985).

Functional Group	Structure	Where Found
Acidic Groups		
Carboxylic acid	$R-CO_2H$	90% of all dissolved organic carbon
Enolic hydrogen	$R-CH=CH-OH$	Aquatic humus
Phenolic OH	$Ar-OH$	Aquatic humus, phenols
Quinone	$Ar=O$	Aquatic humus, quinines
Neutral Groups		
Alcoholic OH	$R-CH_2-OH$	Aquatic humus, sugars
Ether	$R-CH_2-O-CH_2-R$	Aquatic humus
Ketone	$R-C=O(-R)$	Aquatic humus, volatiles, keto-acids
Aldehyde	$R-C=O(-H)$	Sugars
Ester, lactone	$R-C=O(-OR)$	Aquatic humus, tannins, hydroxy acids
Basic Groups		
Amine	$R-CH_2-NH_2$	Amino acids
Amide	$R-C=O(-NH-R)$	Peptides

Fulvic acids also contain more acid functional groups, such as carboxylic acid (COOH). Fulvic acid typically has a higher total acidity compared to humic acid, contributing to its high solubility over all pH ranges. However, humic acids generally contain more phenolic content than FA. Both carboxylic acids (COOH) and phenolic acids (OH) contribute to the observed acidity, with COOH being the most influential. Table 3 lists the average carboxyl and phenolic contents of aquatic humic substances in bogs, marshes and swamps (Malcolm, 1985).

Table 3. Carboxyl and Phenolic content (meq/g) of aquatic humic substances for streams and rivers (Thurman, 1985).

	Carboxyl	Phenolic
Fulvic Acid	5.0-5.5	1.5
Humic Acid	4.0-4.5	2.0

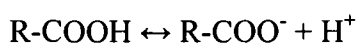
Aquatic humic substances generally contain a carbohydrate content of three percent or less. Previous work has shown that HA has greater carbohydrate content, two to four percent, than FA, which contains less than two percent (Thurman, 1985).

Nitrogen is also a component of both stream humic and fulvic acids. There is approximately one nitrogen atom that makes up one molecule of fulvic acid, but not all fulvic acids contain nitrogen. Stream humic acids contain two to three nitrogen atoms per molecule. The higher amounts of nitrogen found in aquatic humic acids is associated with the greater amino acid content. However, this is only in trace amounts—1 amino acid per 5 humic molecules (Malcolm, 1985).

The acidic functional groups of humic substances are ionized and unionized making the HS polar and/or charged; this also contributes to their solubility (Malcolm, 1985). The ionization potential of humic substances is based on the amounts of acidic groups found within their structure. The average pK_a of humic substances is 4.2. This average is based on (i) various types of acid functional groups found in natural organic matter and (ii) the close proximity of the groups in the organic (humic) molecule. When functional groups ionize (dissociation of H^+), the negative charges generate repulsive forces. This causes the energy of ionization to increase within the carboxyl group, causing the pK_a to increase (Thurman, 1985).

pH plays an important role in determining the ionization of the humic molecules. For example, an aliphatic carboxyl group has a pK_a of 4.8, meaning 50% of the carboxylic groups are dissociated at pH 4.8. When pH conditions are less than the pK_a , the organic molecule is protonated (non-dissociated) when pH conditions are larger than the pK_a the organic molecule is dissociated (ionic) (Dias, personal communication).

There are important implications for the organic molecule and its ability to ionize. First, the solubility of the organic acid increases when it is dissociated:



and

$$K_a = (R-COO^-) (H^+).$$

Second, the hydrogen ion when dissociated contributes to the chemical weathering. The lower the pK_a of an organic molecule the more readily it will dissociate and dissolve Si. And third, the ionic groups are sites for metal complexation (Thurman, 1985). As discussed previously, the humic and fulvic acids are polyprotic acids and their potential and active acidity can play a major role in the pH of natural waters. Therefore, they have the ability to exert a tremendous amount of buffering capacity in streams. In most rivers and streams, with a pH range of 5 to 8, the HA and FA are present as organic polyanions and the majority of this charge is pH dependent; the higher the pH, the more negative the charge, due to the ionization of carboxyl and phenolic functional groups.

Humic Substances—Isolation Techniques

Studies of humic substances and their characterization require fractions free of organic contaminants and other suspended particulate matter found in surface waters.

Therefore, the isolation procedure is an important first step in the study of aquatic humic substances. For major volumes of water, the most common method of isolation is the use of column sorption (Peuravuori, 2002) of macroporous resins (Thurman and Malcolm, 1981). Resins often differ in pore size, surface area, polymer composition and polarity. Resin surface area strongly affects absorption efficiency for lower molecular weight solutes. However, as molecular weight increases, the effect of resin pore size capacity becomes more significant. Pore size also affects the rate of sorption of large molecules (Aiken et al., 1992).

Recent studies have shown that high recovery rates between 50 to 90 percent of organic compounds using non-ionic macroporous sorbents are possible. The most widely used to isolate these substances in fresh, surface and groundwater is a methyl methacrylate resin (Table 4) (Thurman and Malcolm, 1981). However, the most popular non-ionic adsorbent resin, Amberlite™ XAD-8, has not been manufactured for several years. It has been stated from the International Humic Substance Society and scientifically evaluated by Peuravuori et al. (2002) that Supelite™-Supelco DAX-8 (also a methyl methacrylate resin) can be substituted for XAD-8. Although the process used in

Table 4. Properties of XAD-8 resin (Aiken, 1985).

Resin	Composition	Average Pore Diameter, Å	Specific Surface Area (m ² /g)	Specific Pore Volume (cm ³ /g)	Solvent Uptake, g per g of Dry Resin
XAD-8	Acrylic ester	250	140	0.82	1.31-1.36

the manufacturing of these two resins are slightly different, the technical specifications (pore size and surface area) differ little between the two. It has been observed that DAX-8 is easier to handle and its ability to wet is better (Peuravuori, 2002).

The major advantages of this type of separation include its ability to apply the method across a wide range of natural environments and the fractionation process is performed on the original water sample without using a pre-concentration step. Also, the excellent elution efficiencies of the acrylic ester resins for humic substances is due to the charge repulsion between both fulvic and humic acid and the resin because they are anionic at pH 13 (Thurman, and Malcolm, 1985). One of the largest limitations to using resins is the resin must be cleaned very thoroughly in order to keep the DOC bleed low and recovery high (Thurman, 1985). Other types of isolation techniques used in the study of humic substances are listed in Table 5. For this current study, DAX-8 resin column separation was selected due to the large volume of water processed in order to obtain sufficient amounts of HA and FA.

Leenheer (1981) divided organic acids in natural waters into hydrophobic and hydrophilic acid fractions. Aquatic fulvic and humic acid are major components of the hydrophobic fraction. Both portions of DOC adsorb on a column of XAD-8 (and now DAX-8) resin. The hydrophobic acid fraction contains aliphatic, carboxylic acids of 5-9 carbons, one- and two-ring phenols, and aquatic humic substances. The portion of DOC passing through the XAD/DAX-8 resin column as effluent at pH 2, which sorbs onto the XAD-4 resin is considered the hydrophilic acid fraction. This fraction can contain polyfunctional organic acids and aliphatic acids with 5 or fewer carbon atoms. Therefore, the organic acids found in surface waters range from high to low molecular

weight and make-up a complex heterogeneous pool with different behavior on resin sorbents (Aiken et al., 1992). Few studies have been performed on the fraction that is contained on the XAD-4 resin.

Table 5. Advantages and Limitations of various isolation techniques (Thurman, 1985).

Method	Advantages	Limitations
Precipitation	None.	Fractionates sample, not specific for humus, slow on large volumes.
Freeze concentration	All DOC concentrated.	Slow, tedious procedure, concentrates inorganics.
Liquid extraction	Visual color removal.	Not quantified by DOC, slow for large volumes.
Ultrafiltration	Also separates by molecular weight.	Slow.
Strong anion-exchange	Efficient sorption.	Does not desorb completely.
Charcoal	Efficient sorption.	Does not desorb completely.
Weak-anion exchange	Adsorbs and desorbs efficiently.	Resin bleeds DOC.
XAD-resin	Adsorbs and desorbs efficiently.	Resin must be cleaned to keep DOC bleed low.

Humic Substances—Characterization Techniques

Infrared spectroscopy (IR) has been used in the characterization of humic substances, particularly humic and fulvic acids. Application of this type of spectroscopy is used for functional group analysis. This method is quite advantageous for a number of reasons (i) it is non-destructive (ii) only small sample weight is required for analysis (iii)

it is experimentally simple and does not require special manipulative skills or high tech computation and (iv) can provide important information on molecular structure and chemical interactions (Schnitzer and Khan, 1972).

The ability to examine the functionality of the HSs is due to the fact that absorption bands are related to particular vibrations of certain bonds at a given frequency (wavelength, cm^{-1}). The characteristic spectral region from $4,000\text{ cm}^{-1}$ to 1200 cm^{-1} is the range most useful in the study of humic substances. There are approximately eight major absorption bands that can be identified in humic and fulvic acids (MacCarthy and Rice, 1985). The characterization and description of the major absorption bands of Dismal Swamp humic and fulvic acids used in the current study can be found in CHAPTER III Results and Discussion.

Hydroxides and Clay Minerals

Interactions between humic substances, metal hydroxides and clays are a common occurrence in nature. Adsorption of organic compounds onto inorganic colloids can tremendously alter the physicochemical properties of the colloid whose behavior (colloidal stability, transport and interaction with environmental contaminants) may be a product of the adsorbed DOM (Zhou et al., 2001).

Fe-hydroxides (goethite) and Al-hydroxides (gibbsite) are hydrous oxides characterized by the presence of a hydroxyl (OH^-) groups. The presence of OH^- groups results in weaker bond strengths compared to their associated mineral oxides. They are unlike layered silicate clays in that they do not develop structural charge from isomorphous substitution. As a result, they have low cation exchange capacities. On

average, both goethite and gibbsite have cation exchange capacities ranging from 2 to 8 cmols kg⁻¹. However, surface layer charge can form based on the pH of the surrounding solution (McBride, 1994).

Upon comparison of the physical properties of gibbsite and goethite, one can see these two hydroxides have similar interlayer spacings, yet their external surface areas are drastically different (Table 6). The external surface area could therefore result in differences in the adsorption capacity of humic substances along with other organic compounds.

Table 6. Physical properties of gibbsite and goethite (Brady and Weil, 2002).

Colloid	Type	Size, μm	Shape	Surface Area (m ² /g)		Interlayer Spacing ^a , nm	Net charge ^b , cmol _c /kg
				External	Internal		
Gibbsite	Al-hydroxide	<0.1-5.0	Hexagonal Crystals	5-30	--	0.48	-1 to -15
Goethite	Fe-hydroxide	<0.1	Variable	100-300	--	0.42	+20 to -5

^a From the top of one layer to the next similar layer 1nm = 10⁻⁹ m = 10Å; ^b Measure of ion-exchange capacity

Aluminum hydroxide (AL) typically reacts as an adsorbent in a pH range from 3 to 9 and has a p_H_{pzc} 9. At pH > 9, the overall net surface charge is negative and will not bind organic acids. At pH < 9, it accepts protons from solution to form a positively-charged surface. Iron hydroxide (FE) has an iso-electric point that ranges from 7.0 to 8.4 and maximum adsorption of organic matter occurs at pHs between 5 and 6. The surface is positively charged over the pH range 3-9. The onset of adsorbing organic matter causes the surfaces of the alumina and iron to have a negative charge (Thurman, 1985).

Humic substances contain a wide range of acidic functional groups, which have a high specificity for AL and FE complexation. Two reactions controlling the ability for the hydroxides to complex with these acidic groups are (1) The ease of dissociation between the functional groups. Carboxylic groups have a low pK_a (acid dissociation) and dissociate readily compared to phenolic groups. (2) The strength of the functional group— Al^{3+} or Fe^{3+} bond. This is controlled by the electron-donating power of the acid group ligand (McBride, 1994).

Adsorption mechanisms that have been proposed involve (i) anion exchange (electrostatic interaction), (ii) ligand exchange-surface complexation, (iii) hydrophobic interaction, (iv) entropic effect, (v) hydrogen bonding and (vi) cation bridging. The differences in procedures and experimental conditions used and the heterogeneity and complexity of the OM and adsorbent surfaces account for the inconsistencies among individuals (Gu et al., 1994).

Studies have shown that pH was the most influential factor involved in adsorption when temperature, pressure and initial concentration of the adsorbate were held constant (Krauskopf and Bird, 1995). Adsorption of humic substances to FE and AL is highly dependent on pH. Therefore, it would seem likely that this observed dependency is consistent with the anion exchange (or electrostatic interaction) mechanism, since the surfaces of the hydroxides and humic substances are positively and negatively charged. However, based on studies and reasoning of Gu et al. (1994) it was not considered to be the major mechanism in adsorption.

Alumina

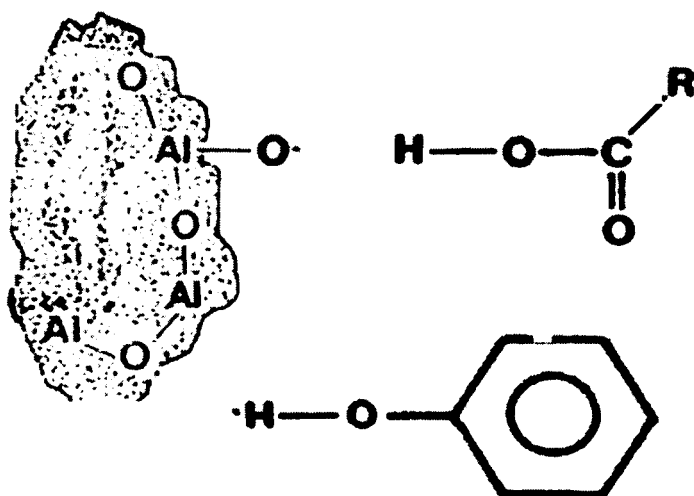


Fig. 4. Illustration describing the bonding of organic matter to alumina (Thurman, 1985).

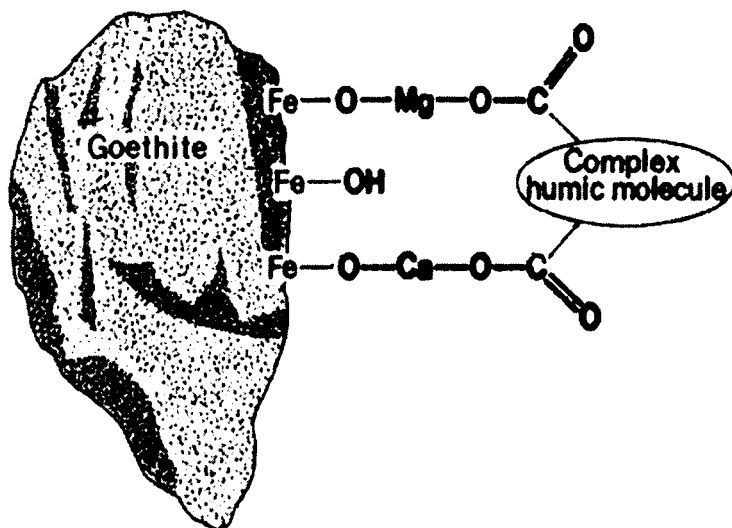
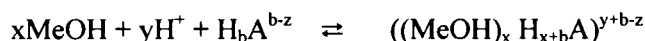


Fig. 5. Proposed model of DOM sorption onto goethite through cation bridging (Thurman, 1985).

Gu et al. (1994, 1995) and Davis (1982), among others, have proposed that the mechanism of sorption of organic matter onto alumina (Fig. 4) and Fe surfaces (Fig. 5) takes place by complex formation-ligand exchange reactions (chemisorption). The increase in OM adsorption with decreasing pH is consistent with this type of mechanism. The reaction occurs between the protonated mineral-surface hydroxyls and carboxyl or phenolic groups of the organic molecule. Ligand exchange is distinguished from anion exchange due to (i) release of OH^- into solution (ii) a high degree of specificity shown toward particular anions (iii) a tendency to be nonreversible/desorption slower than adsorption and (iv) a change in surface charge to a negative value (McBride, 1994).

Davis (1982) described the sorption reaction of Al (Fig. 4) using the following equation:



where MeOH is the metal hydroxide, H are protons and HA is the carboxyl group of aquatic fulvic acid. This conventional notation is based on charge calculation on a unit cell basis, where the colloid is composed of an assemblage of unit cells. He explained, the alumina surface is relatively basic, accepting protons from solution (at pH values less than 9) to form a positively-charged surface. Thus, the tendency for complex formation between the acidic function groups of organic molecules is high with the more basic alumina hydroxyls compared to a silica surface, which is acidic and releases protons from its surface hydroxyls.

The reactions occurring between OM and Fe-OH_2^+ and Fe-OH surface groups cause H_2O and OH^- displacement. Below pH of ~ 8 , protonation of the surface hydroxyls on the Fe hydroxide render the surface hydroxyls more exchangeable, contributing to the

pH dependent adsorption of the organic ligand (Gu et al., 1995). Possible modes of ligand exchange interactions between iron and OM were postulated analogous to the binding of Fe^{3+} with humic or fulvic acids. The equations below illustrate the displacement of OH and H_2O from the surface of the oxide (Gu et al., 1994):



Processes involved in physical weathering reduce particle size of mineral grains, increasing the exposed surface area. Chemical weathering processes produce more significant changes, dissolving or altering the rock-forming minerals called primary minerals to produce layer silicate (clay minerals) and oxide minerals. These products of weathering are referred to as secondary minerals. Clay minerals are phyllosilicates, *i.e.* silicates having a platy sheet structure. Physical and chemical properties of both kaolinite and smectite are listed in Table 7.

Kaolinite is probably the most common aluminosilicate mineral found in soils and permeable bed rock, formed as a residual weathering product from feldspars, that

Table 7. Physical properties of kaolinite and smectite (Brady and Weil, 2002).

Colloid	Type	Size, μm	Shape	Surface Area (m^2/g)		Interlayer Spacing ^a , nm	Net charge ^b , cmol _c /kg
				External	Internal		
Smectite	2:1 silicate	0.01-1.0	Flakes	80-150	550-650	1.0-2.0	-80 to -150
Kaolinite	1:1 silicate	0.1-5.0	Hexagonal Crystals	5-30	--	0.72	-1 to -15

^a From the top of one layer to the next similar layer $1\text{nm} = 10^{-9}\text{m} = 10\text{\AA}$ ^b Measure of ion-exchange capacity

eventually weathers to Al-(hydr)oxide. Kaolinite, having a 1:1 layer type, is made up of alternating aluminum octahedral and silicon tetrahedral sheets. The general formula for kaolinite is $\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$ and from this formula there is no recognized substitution in most species. Therefore, no layer charge or very small layer charge exists (Moore and Reynolds, 1997).

Because of the crystal structure arrangement and the position of the octahedral and tetrahedral sheets, a plane of oxygen atoms on the bottom surface and a plane of hydroxyls on the upper surface of the clay micelle are exposed. This arrangement has two important implications. First, where the hydroxyls are exposed on the clay surface, removal or addition of hydrogen can create positive or negative surface charges. This is pH controlled and can influence the type of ions or organic molecules (functional groups) sorbed onto the surface. Second, the 1:1 structure facilitates hydrogen bonding in between the octahedral and tetrahedral sheets. Because of the interlayer hydrogen bonding, kaolinite has neither expansion capabilities nor internal surface area (Brady and Weil, 2002).

Smectite is a classification for a group of minerals (Montmorillonite being the most prominent) all of which display the ability to expand and contract their structures. These clay minerals have a 2:1 structure characterized by one octahedral sheet in between two tetrahedral sheets. The addition of water causes the clay mineral to expand and an interlayer cation can be replaced by another cation. Therefore, the cation-exchange capacities are high compared to the non-expanding clay minerals, such as kaolinite (McBride, 1994).

The smectite group has a permanent negative charge, which is a product of isomorphous substitution occurring primarily in the octahedral sheets. Most of the negative charge of smectite is caused by Mg^{2+} ions substituted in the octahedral Al^{3+} positions (Brady and Weil, 2002). Thus an ideal formula for a montmorillonite micelle is $\text{R}_{0.33}^{+}(\text{Al}_{1.67}\text{Mg}_{0.33})\text{Si}_4\text{O}_{10}(\text{OH})_2$ where R is the exchangeable cation in the interlayer space (Moore and Reynolds, 1997). The isomorphous substitutions associated with the smectite group account for their high capacity to adsorb cations.

Smectite has a higher external and internal surface area compared to kaolinite. The negative net charge of smectite is also higher (more negative) relative to kaolinite (Brady and Weil, 2002). The properties listed above enable clay minerals to bind and chemically stabilize organic matter.

Natural clays are most probably amorphous on the outer surfaces due to the continuous and natural processes of partial dissolution and reprecipitation of clay components at the solid-aqueous interface. These processes affect the exposed edges, planar and interplanar sites on clays at varying pH. The implications of these observations are that the adsorption of compounds (organic and inorganic) is dependent on the type of surfaces exposed to the liquid phase (Schulthess and Sparks, 1989).

As colloids, clay minerals occur in association with humic material as a clay-metal-humic complex. Clay minerals and organic colloids have negatively charged sites and within the natural environment, positively charged cations (M^{2+} , M^{3+}) serve to neutralize the charge and bridge the two together (Fig. 3). The main polyvalent cations responsible for this binding are Ca^{2+} , Fe^{3+} , and Al^{3+} (Stevenson, 1985).

Numerous studies have examined the sorption of OM on secondary minerals, such as goethite and kaolinite. These studies focus on changes in molecular weight distribution and adsorption isotherms (Zhou et al., 2001; Meir et al., 1999). Both studies found that greater amounts of NOM were sorbed onto goethite than kaolinite. Namjesnik-Dejanovic et al. (2000) examined the two minerals' adsorption isotherms and found the shapes of the two curves were dramatically different, perhaps because of the distribution and concentrations of surface reactive hydroxyl sites. The isotherm of goethite showed a gradual decrease in % adsorption, while kaolinite showed a steep initial drop-off. Meir et al. (1999) suggested several factors responsible for those results. First, if some sorption is electrostatically driven then the greater pH_{pznpc} (pH of point of zero proton condition) of goethite (7.7) than kaolinite (5.3) may play a role. At the pH of these experiments (4), most of the NOM has a net negative charge and the goethite has a positive charge, while kaolinite is closer to neutral and its edge sites are positively charged. More importantly, the chief mechanism of sorption of OM to mineral surfaces is through ligand-exchange at surface hydroxyl sites. Thus, the density and position of the hydroxyl sites on the surfaces of goethite and kaolinite must be considered important factors on their differences in sorption. Goethite, which is typically rod shaped when prepared in the laboratory using nitrate salts, has a higher density of hydroxyl groups spread throughout its surface. Conversely, the hydroxyl sites (octahedral aluminum groups) on the surface of kaolinite are specifically located at the edges of its crystal structure. Third, there may be a lesser affinity for Al-versus Fe-centered sorption sites and sorption is minimal on Si-centered sites of kaolinite. Kerndorf and Schnitzer (1980) supported this data from studying the competitive sorption of metals to HA in aqueous

solution and found that Fe out-competed Al. They stated that if surface complexation is comparable to solution binding, then by analogy, the greater adsorption to goethite than kaolinite and possibly Al-hydroxide could be related to the relative affinities for Fe versus Al-centered sites.

Gu et al. (1995) investigated the adsorption/desorption of NOM on Fe oxide. The NOM pool was divided into the hydrophobic (HbA), hydrophilic (HL), <3K and >3K fractions. They found that the adsorption of HbA and the >3K fractions of NOM were consistently higher over the entire range of pH. This indicated, based on C, larger molecular size HbA fractions of NOM are preferentially sorbed by iron oxide over the smaller molecular size HL fractions. They also found that carboxyl functional groups of NOM might contribute more than hydroxyl groups in measuring NOM sorption, unless the hydroxyl is ortho-positioned with respect to the carboxyl groups or the experiment is carried out at high pH conditions.

Experimental Objectives

The objectives of this research project are to 1) Examine, by IR spectroscopy, the spectral pattern differences existing between Dismal Swamp humic and fulvic acids. Evaluating those differences was accomplished by characterizing the specific functional groups of the individual HA and FA freeze-dried samples. 2) Examine, by IR spectroscopy, any spectral change of HA/FA after adsorption onto Fe-hydroxide, Al-hydroxide, Kaolinite and Smectite. The portions of HA and FA that were not adsorbed onto the four mineral colloids were freeze-dried and analyzed. In other words, the HA and FA fractions remaining in solution (not sorbed) were examined. 3) Compare

similarities and differences of the four mineral colloids in regards to relative amounts of HA or FA sorbed, measured by DOC analysis in mg C L^{-1} . The amount of HA and FA sorbed in mg C L^{-1} was then converted to mg HA/FA m^{-2} for each colloid. 4) Compare similarities and differences of the FA and HA IR spectra after adsorption considering those responses after exposure to the four mineral colloids. The following questions were addressed from the previous objectives:

1. Does HA or FA show preferential sorption to one of the four mineral colloids?
2. Will more adsorption of HA or FA occur with the hydroxides than the clay minerals because of a higher number of surface OH sites?
3. Because kaolinite and smectite are clay minerals and share similar compositional units, which clay will adsorb more FA or HA?
4. Since AL is similar to the octahedral layer of kaolinite, will similar adsorption to HA or FA occur?
5. Will more adsorption of HA occur based on its higher molecular weight compared to FA?

CHAPTER II

METHODS

This project was designed to explore the nature of binding between organic matter and inorganic colloids by examining the functionality of humic and fulvic acids before and after adsorption using FT-IR. A flow chart of the experimental design is shown in Figure 6. Experiments were divided into three sections: (1) Colloidal Synthesis, (2) Isolation of humic and fulvic acids and (3) Adsorption/Equilibrium studies.

Colloidal Synthesis

The Fe hydroxide (FE) and Al hydroxide (AL) were prepared in the laboratory via precipitation reactions. The FE was prepared by adding 5 M KOH to 1 M $\text{Fe}(\text{NO}_3)_3$ and diluting to 2 L with NANOpure DI H_2O according to the methods of Schwertmann and Cornell (1991). The reddish brown Fe suspension was placed in the oven in a covered polyethylene beaker at 70°C for approximately 60 hours where it was converted to a yellow precipitate of goethite. The AL was made using 1M $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 5 M KOH based on the methods of Kinniburgh et al. (1975). The pH of the Al hydroxide solution was significant in promoting precipitation. The pH of a 1 M solution of $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was monitored using an Orion research digital ionanalyzer 501 and 5 M KOH was added until pH 6.5 was obtained. The pH probe was calibrated two times daily using pH 4 buffer solution. During this process a cloudy white precipitate of AL

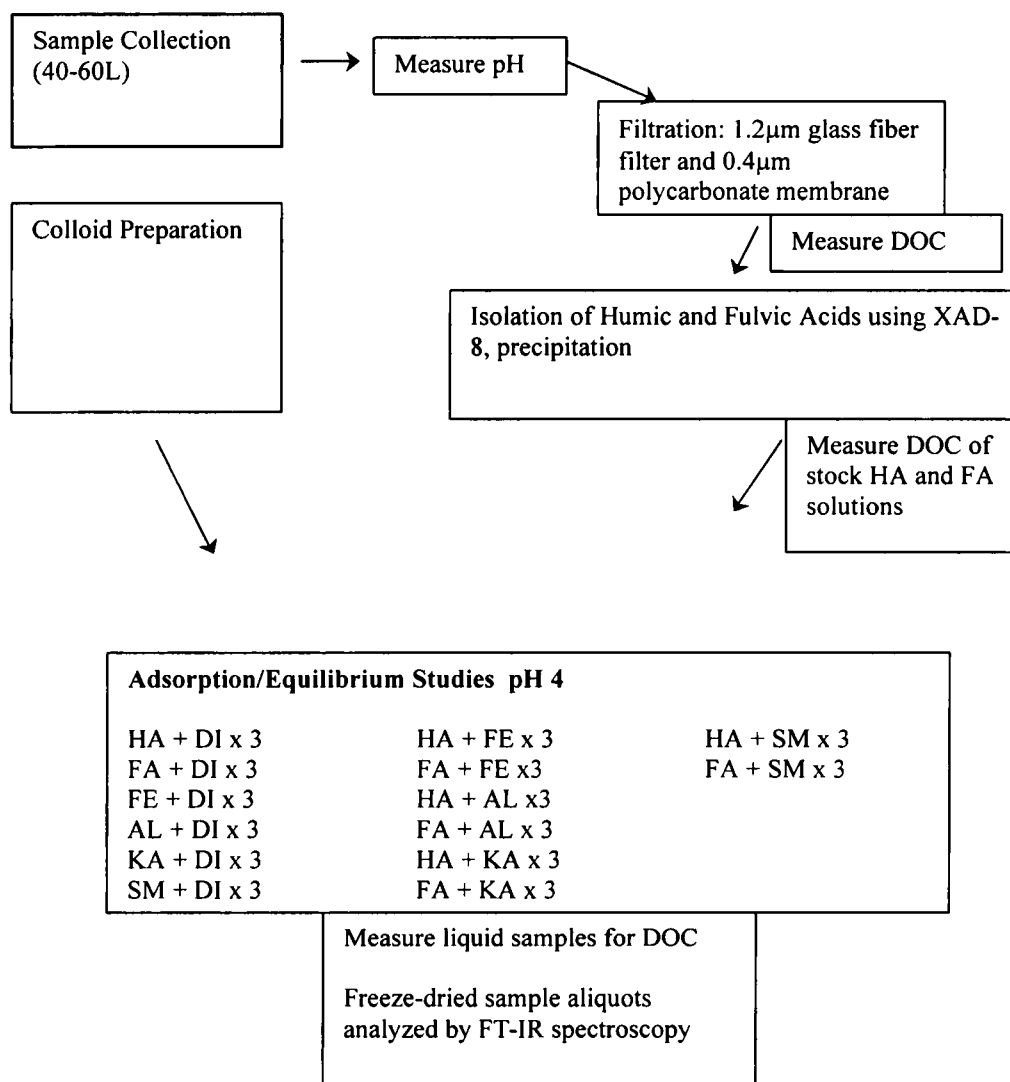


Fig. 6. Experimental design flow chart.

formed. The suspension was placed in the oven in a covered polyethylene beaker at 70° C for approximately 60 hours. After removal from the oven, each suspension was washed to remove any nitrate remaining, centrifuged (Beckman, Model J2-21), and lyophilized using a HETO FD4 freeze-drier to obtain a solid phase.

The less than 2µm size fractions of kaolinite (KA) and smectite (SM) were recovered based on the particle settling technique from Jackson (1969). The clay minerals were ground using a mortar and pestle then placed in 250 mL plastic bottles with dilute sodium hexametaphosphate. The bottles were shaken on an end-to-end shaker for 10-20 minutes. The bottles were removed from the shaker and allowed to sit undisturbed for 5 minutes to allow coarse silt to settle. The suspension was decanted into a 4 L glass beaker. This process was repeated until the clay and fine silt were separated from the coarser material. Particle settling rates were calculated for 2 µm clay particles and at the end of the settling period, clay remaining in suspension was siphoned using glass tubing with an upward curve into a 10 L glass carboy. 1 M MgCl₂ was added to the clay suspension to promote flocculation. Once the clay had settled, the clear liquid was removed and discarded. The volume was reduced via centrifugation and then lyophilized (freeze-dried) to obtain a solid phase. The detailed procedures of the colloids are summarized in Appendix A.

Specific surface area was determined on all four colloids using a CaCl₂–ethylene-glycol solvation technique from the method of Carter et al. (1986). The surface area of the colloids was calculated based on the amount of ethylene glycol monoethyl ether (EGME) absorbed. A detailed explanation can be found in Appendix B. Fe-hydroxide, Al-hydroxide and kaolinite were chosen for this experiment due to their ubiquitous

nature. More importantly, they are commonly found within the Coastal Plain as suspended colloids in local river systems. Smectite was chosen because it is found in other parts of the state and is a different type of clay mineral end-member when compared to kaolinite.

Humic and Fulvic Acid Separation

Aquatic samples (60 L) were collected in glass carboys from the Great Dismal Swamp Wildlife Refuge in Chesapeake, VA. The collection site was slightly inland at the intersection of Jericho Ditch and Lake Drummond. The pH of the water sample was measured on site. An aliquot of the water sample was taken for analysis of DOC concentration. The 60 L of sample was stored no more than a week at 4° C in the dark. Incorporation of a prefiltration step using cleaned 1.2 µm Gelman type A/E glass fiber filters aided in faster passage through a 0.4 µm polycarbonate membrane filter. The humic (HA) and fulvic (FA) acids were separated using a resin isolation technique developed by Thurman and Malcolm (1981) (see Appendix D). The resin was obtained from Supelco (Supelite™ DAX-8) and fines were removed initially. The beads were rinsed with NaOH and then in acetonitrile, methanol, and DI H₂O. Because this was new resin, it was not placed in a soxhlet extractor. Glass columns were packed with H₂O-resin slurry and rinsed with DI H₂O to remove any remaining methanol. The column was further cleaned with 3 successive 0.1 N NaOH—0.1 N HCl rinses immediately before using during the entire separation process. The cleaning and packing procedure is outlined in Appendix C. After filtration, the sample was acidified to pH 2 and passed through a packed glass column of clean DAX-8 resin using a Masterflex™ peristaltic

pump (Model 7518-60) at a flow rate of 10 bed volumes per hour. Bed volume was calculated as $V_c = \pi r^2 L$, where r and L are the radius and length of the resin within the column. The HA and FA sorbed onto the resin were eluted in reverse direction with 0.1 N NaOH at a flow rate of 5 bed volumes per hour and immediately acidified to pH 2 to avoid oxidation of the humic substances. The eluate was reconcentrated on a smaller DAX-8 column and eluted in reverse order with 0.1 N NaOH. To promote precipitation of HA, the pH of the eluate was adjusted to 1 with concentrated HCl and stored for 24 hours at 4° C in the dark. The HA and FA were placed in polypropylene centrifuge tubes and centrifuged for 30 minutes at 10,000 rpm to settle the HA precipitate. The HA was rinsed with DI water until the AgNO_3 test showed no Cl^- in wash-water. Then, the HA was dissolved in 0.1 N NaOH and hydrogen saturated by passing the solution through a column of Bio-Rad® cation exchange resin AG-50W-8X 100-200 mesh in H^+ -form. The FA suspension was filtered with a Teflon 47 mm 0.2 μm pore size filter to remove the large floccules of HA still present. The fulvic acid was reappplied at pH 2 to a smaller DAX-8 column and desalted by rinsing the column with 1-void volume of DI water to remove HCl and inorganic salts. The fulvic acid was eluted in reverse direction with 0.1 N NaOH and immediately passed through a cation exchange resin column in H^+ form. The HA and FA were then lyophilized.

Adsorption/Equilibrium study

Adsorption studies were conducted in a manner similar to Zhou et al. (2001) and Gu et al. (1994). Stock suspensions of all four colloids and the two organic acids (stored at 4° C in the dark) were prepared by adding freeze-dried solid to DI H_2O one day prior to

the experiment, permitting full hydration and adjusted to pH 4 using concentrated HCl. Concentrations of stock suspensions were prepared based on the specific surface areas of each colloid in order for the total surface area to solution ratio to remain consistent throughout the experiment. Fe-hydroxide and kaolinite showed similar surface areas, $76.8 \text{ m}^2/\text{g}$ and $75.2 \text{ m}^2/\text{g}$, and the stock suspension concentration of each was 2 g/L . Al-hydroxide and smectite showed similar surface areas, $832.0 \text{ m}^2/\text{g}$ and $826.7 \text{ m}^2/\text{g}$, and the stock suspension concentration was 0.5 g/L . On average, the exposed surface for all four colloids was 0.800 m^2 . Concentration of the HA and FA suspensions was 60 mg/L . Aliquots of both HA and FA were taken and analyzed for DOC concentration. 0.1 M NaCl was added to the four mineral colloids so that the final background electrolyte concentration was 0.01 M NaCl during the adsorption process. The following day, the stock suspensions were adjusted to pH 4 using 6 M NaOH and HCl. Next, 35 mL of FA or HA were added to 40 mL polycarbonate centrifuge tubes. 5 mL of the kaolinite and Fe-hydroxide stock suspensions and 2 mL of the Al-hydroxide and smectite stock suspensions were added to the centrifuge tubes containing each organic acid. Two types of control samples were also prepared, HA and FA with no colloid (DI H_2O substituting for colloid) and the mineral colloid with no organic acid (DI H_2O substituting for organic acids).

After shaking for 24 hours in the dark, samples were centrifuged for 30 minutes at $12,000 \text{ rpm}$. The supernatant was collected and filtered through a $0.1 \text{ }\mu\text{m}$ Millipore polycarbonate membrane. The filtrate (the portions of HA and FA not sorbed) was split into different glass vials for DOC and FTIR analysis. DOC samples were placed in the -20°C freezer until the analysis was performed. DOC was analyzed in triplicate on a

Shimadzu TOC-5000 analyzer. The amount of HA or FA adsorbed onto each colloid was calculated from the difference between the initial (before adsorption) and the final (after adsorption) DOC. The HA and FA blank control samples were the initial DOC concentrations used in calculating amounts sorbed.

DOC concentrations were analyzed using a 2-way analysis of variance (ANOVA) model with interaction and two factors. Factor 1 was comprised of four categories: FE, AL, KA and SM. Factor 2 consisted of two categories: HA and FA. The model was set up as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where Y is the response of the DOC data, μ is the common mean, α is the effect of the colloids, β is the effect of the organic acids, $(\alpha\beta)$ is the interaction effect and ε is random error. The subscripts indicate that: k is the number of replications (3) per sample, j represents the acids, and i reflects the colloids. This analysis also aided in outlier detection. The model was executed in SAS and Microsoft Excel was used to set up the spreadsheets for the analysis.

The samples undergoing FTIR spectroscopy were placed in the freeze-drier in order to prepare pressed KBr pellets. KBr pellets were prepared by pressing approximately 0.5-1 mg of sample (depended on recovery) and 50-100 mg of IR grade KBr at 15,000 kg/cm² pressure on a Carver hydraulic press for 1 minute. Functionality of the organic acids after adsorption was examined using a Nicolet 5PC FT-IR Spectrometer equipped with Omnic program version 3.1a for collecting and saving spectra. Spectra were collected at a resolution of 4 cm⁻¹ with 64 scans per run from 4000 cm⁻¹ to 400 cm⁻¹. One single spectrum was obtained by combining all three replicates into one. Then, a

smoothing command within the program was performed on the spectra, which improved their appearance and clarified real peak shapes that had been obscured by background noise. The smoothing command within the computer program uses the Savitsky-Golay algorithm (Omnic version 3.1a). Generally, spectra were smoothed by 11 points.

Spectra were normalized in order for comparison between the colloids and organic acid adsorption spectral peaks. Raw spectral data was obtained and imported into a Microsoft Excel spreadsheet. The minimum absorption value was subtracted from all absorption values, making the minimum value 0 and a set of new absorption values. The new maximum was identified and then divided into the new absorption values, setting the maximum value to equal 1. Once the spectra were normalized, peaks associated with the functional groups of the organic acids were characterized. Comparisons were made between the initial HA and FA spectra and post adsorption spectra of the FA and HA (not sorbed) in relation to each acid and the four colloids.

CHAPTER III

RESULTS AND DISSCUSSION

Fourier Transform Infrared (FTIR) spectroscopy was performed on freeze-dried Dismal Swamp humic and fulvic acids for functional group analysis using pressed KBr pellets. These spectral patterns are very similar to one another (Fig. 6), as well as other published spectra reported in Senesi et al. (1989) and Schnitzer and Khan (1972). Detailed interpretation of infrared spectra can be quite challenging due to the overlapping of individual absorption bands at different vibration modes by different functional groups. Difficulty occurs with identifying the functionality of humic and fulvic acids due to their complex molecular structure (Chen et al., 2002)

Characterization of original FA and HA

The normalized IR spectral patterns of the Dismal Swamp FA and HA are shown in Fig. 7. The absorption peaks that were identified and the corresponding functional group assignments are shown in Table 8. The interpretation of the IR spectra is based on published data from Francioso et al. (2001), Gu et al. (1994), Celi et al. (1997), Senesi et al. (1989), and Chen et al. (2002).

The peak at 2300 cm^{-1} is due to atmospheric CO_2 within the sample holding chamber of the spectrophotometer. The carboxyl peak was more intense in the FA (1721 cm^{-1}) compared to the corresponding peak of the HA. The 1034 cm^{-1} and the 913 cm^{-1} were two peaks identified in the HA that were not evident in the FA.

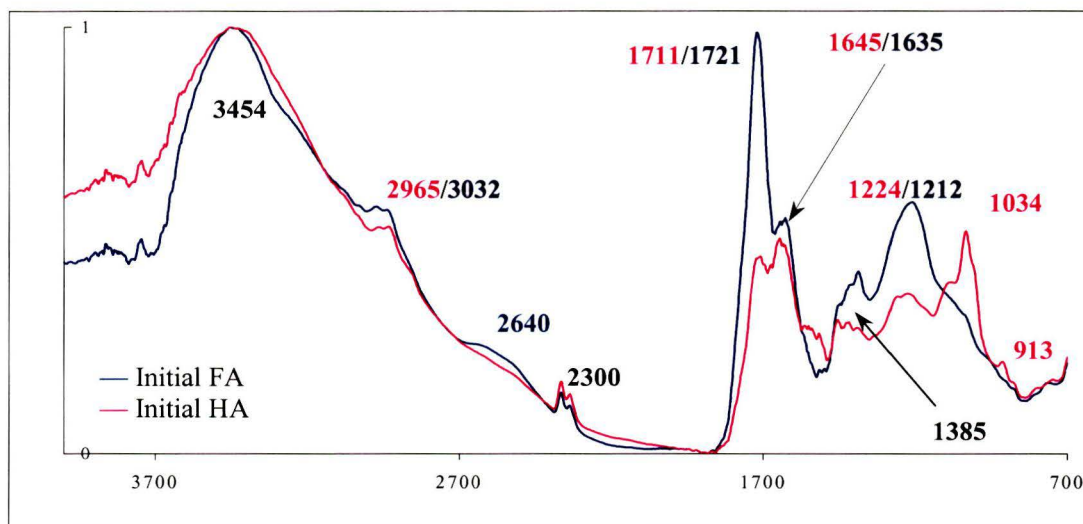


Fig. 7. Initial FTIR spectra of Dismal Swamp humic and fulvic acids.

Table 8. Peak analysis of original Dismal Swamp humic and fulvic acids.

¹Francioso et al. (2001), ²Gu et al. (1994), ³Celi et al. (1997), ⁴Senesi et al. (1989), and ⁵Chen et al. (2002).

Humic Acid Peak (cm ⁻¹)	Fulvic Acid Peak (cm ⁻¹)	Functional Group
3454	3454	Stretching motion of H-bonded OH from carboxylic (COOH) and alcoholic(COH) groups ¹
2965-2933	3032-2939	Stretch of aliphatic C-H from -CH, -CH ₂ , and -CH ₃ groups ²
	2640	OH Stretch from H bonded COOH groups ¹
1711	1721	Stretch of C=O from COOH ³
1645	1635	Asymmetrical stretching of COO ⁻ Aromatic C=C Vibration of carbonyl C=O conjugated with other bonds ⁴
1515	1515	Vibrations of C=O of amide groups (amide II band) ⁴
1453	1447	Bend of aliphatic -CH ₃ ¹
1423	1423	In-plane OH bending of carboxylic acids ⁴
1385	1385	Symmetrical stretching of COO- Deformation of OH Stretching of C-O of phenolic groups ⁵
1224	1212	Asymmetrical stretch of CO Bending of OH from COOH C-OH bending of phenols and tertiary alcohols ³
1034		Stretching of alcoholic and polysaccharide of CO Deformation of OH Si-OH silicate impurities ⁴
913		Out of plane bending of aromatic C-H Vibrations of aliphatic -CH ₂ chains ⁴

Post adsorption characterization of control FA and HA

After the 24-hour equilibrium period control samples (organic acid plus DI H₂O) were freeze-dried and analyzed using FTIR. Both spectra of control FA and HA (Fig. 8 and 9) show very different spectral patterns when compared to their corresponding original patterns (Fig. 7). The absorption bands and the changes they underwent are shown in Table 9.

The original 1721 cm⁻¹ peak shows a significant decrease in intensity in the control FA sample, while the corresponding peak of the HA control sample completely disappeared. Undissociated carboxyl groups (COOH) are related to the band at 1721 cm⁻¹ and carboxylate anion groups (COO⁻) at 1635 cm⁻¹ (Gu et al., 1995). These two peaks, therefore, are very important in identifying the degree of dissociation of carboxyl groups within the humic substances.

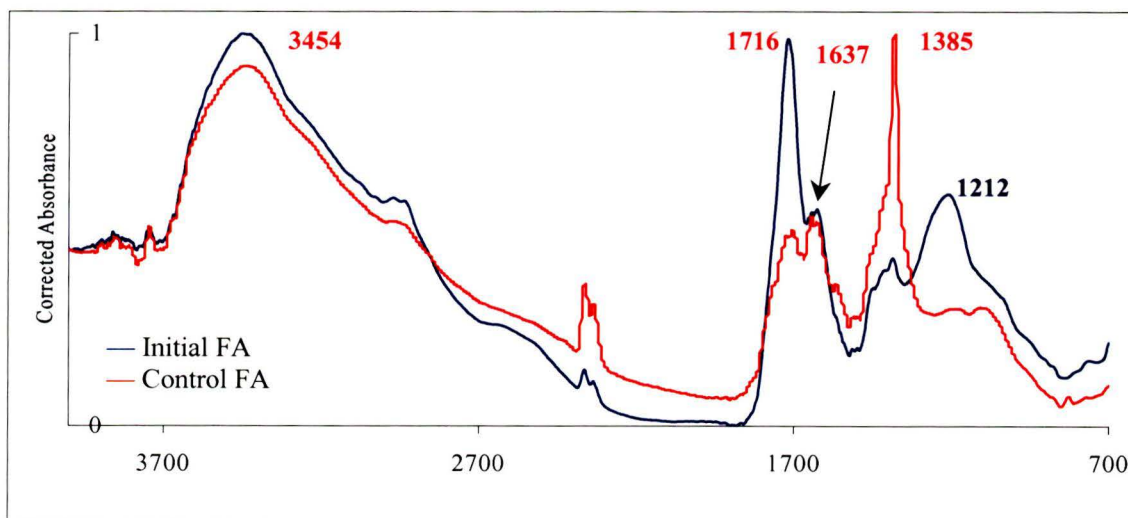


Fig. 8. FTIR absorbance spectra of the original and control fulvic acids.

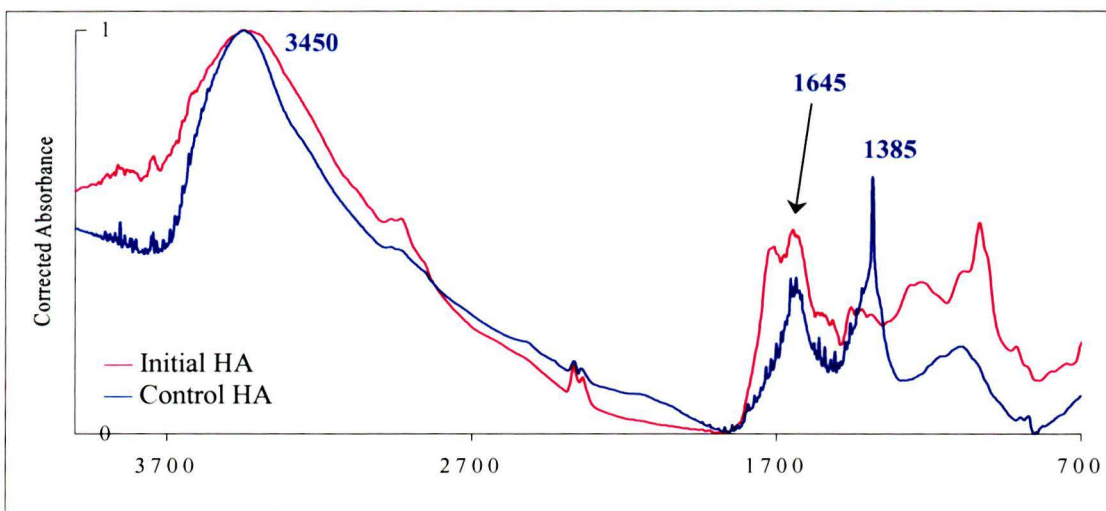


Fig. 9. FTIR absorbance spectra of the original and control humic acids.

The 1385cm^{-1} peaks of both the HA and FA control samples, show a significant increase in intensity. This is probably due to an enrichment of aliphatic or carbohydrate OH-functional groups (Gu et al., 1995), dissociated COO^- , or stretching of C—O of phenolic groups which had been concealed in the original sample due to the 1700cm^{-1} peak. This increase corresponds with the observed decrease in the 1700cm^{-1} peaks (loss of undissociated carboxyl groups). Nevertheless, the functional groups associated with the 1385 peak are numerous and the increase in intensity could be due to a suite of possibilities. The 1212cm^{-1} peak of the FA diminished and very two small peaks emerged at 1250cm^{-1} and 1110cm^{-1} . The two emerged bands could be related to OH bending of phenolic groups, which corresponds with the increased intensity of the HA and FA 1385cm^{-1} peaks.

Table 9. Peak analysis after 24-hour blank equilibrium period. ¹Francioso et al. (2001), ²Gu et al. (1994), ³Celi et al. (1997), ⁴Senesi et al. (1989), and ⁵Chen et al. (2002).

Original Humic Acid	Control Humic Acid	Observed changes	Original Fulvic Acid	Control Fulvic Acid	Observed changes	Functional Group
3454	3450	became less broad	3454	3454	no apparent change	Stretching motion of H-bonded OH from carboxylic (COOH) and alcoholic(COH) groups ¹
2965-2933	2933	shoulder much less apparent	3032-2939	3032-2939	much less apparent shoulder	Stretch of aliphatic C-H from -CH, -CH ₂ , and -CH ₃ groups ²
			2640	--	shoulder disappeared	
1711	--	peak completely disappeared	1721	1716	intensity dropped significantly	Stretch of C=O from COOH ³
1645	1645	no change	1635	1637	no change	Asymmetrical stretching of COO ⁻ Aromatic C=C Vibration of carbonyl C=O conjugated with other bonds ⁴
1515	--	mixed into noise	1515	--	mixed into noise	Vibrations of C=O of amide groups (amide II band) ⁴
1453	--	mixed into noise	1447		mixed into noise	Bend of aliphatic -CH ₃ ¹
1423	--	mixed into noise	1423		mixed into noise	In-plane OH bending of carboxylic acids ¹
1385	1385	significantly increased in intensity; masked the two previous peaks	1385	1384	significantly increased in intensity	Symmetrical stretching of COO- Deformation of OH Stretching of C-O of phenolic groups ⁵
1224	--	disappeared	1212	--	disappeared	Asymmetrical stretch of CO Bending of OH from COOH C-OH bending of phenols and tertiary alcohols ³
1034	1110	decreased in intensity, became very broad		1250 and 1110	became two small humps located where the 1212 peak used to occupy	Stretching of alcoholic and polysaccharide of CO Deformation of OH Si-OH silicate impurities ⁴
913	913	same				Out of plane bending of aromatic C-H and Vibrations of aliphatic -CH ₂ chains ⁴

The observed changes, therefore, for both the HA and FA control samples were a decrease in undissociated carboxylic group (1700cm^{-1}) intensity and an increase in carboxylate (dissociated carboxyl) and phenolic OH group (1385cm^{-1}) intensity. The decrease in intensity of the undissociated carboxyl groups indicates that the humic substances became dissociated during either the stock solution re-hydration period, during the 24-hour equilibrium period, or a combination of both. The changes in protonation state occurred because the pH of the experiment was very close to the pK_a of humic substances (4.2).

Reflecting on the procedures outlined in the adsorption experiment, the differences that existed between the original and control sample handling were the re-hydration of the lyophilized HA and FA for the stock solutions and the filtration step. It was hypothesized that residual material from the filter, even after it had been cleaned, may have been incorporated into the sample, thus causing differences in the original versus control spectral patterns to exist. Most filters available today are composed of some organic material, the exceptions being glass fiber and the highly expensive silver membrane. Therefore, filters have the capability of releasing DOM (Zsolnay, 2003). The purpose of the filtration step used in this experiment was to remove any remaining colloid left after centrifugation. Zhou et al. (2001) found that unfiltered samples contained 2-3% fine goethite particles still remaining in the supernatant. Without removing these fine sized particles, certain analysis, such as DOC, UV/VIS or FTIR, could be biased. They found control samples undergoing filtration showed no significant difference in DOC concentration, ϵ_{280} values, molecular weight or molecular weight distribution in comparison to the unfiltered control samples.

Zsolnay (2003) after extensive laboratory experiments on polycarbonate, cellulose and acetate filters found polycarbonate filters neither adsorbed nor released DOM. Therefore, the polycarbonate filters probably did not contribute to any added DOM into the current samples. For this present study, the mineral DI blanks contained on average 1.7mg C L^{-1} , which would not be a significant contribution to the changes observed between the original versus control samples. Contrary to published findings, a concern arose from this project in regards to changes in spectral patterns that may occur due to the added filtration step. FTIR spectral change of HA and FA before and after filtration has not been investigated.

Examining this matter further, FTIR was run on filters directly from their container and those that went through the cleaning process. The analysis found that peaks from the filters did not correspond with either spectrum of the control samples. FTIR was also run on sodium carbonate to examine the resulting peaks of the bonds between sodium and carbon and oxygen as a possible interference from the Na-background electrolyte solution. The same type of conclusion resulted, in which the peaks did not correspond with the HA and FA control patterns. Therefore, the experimentation provides evidence that the filters did not contribute to the observed change. The matter remains unresolved and warrants further investigation.

The humic substances used in this project were isolated using macroporous resins in order to obtain sufficient amounts for the experiment. Ghosh and Schnitzer (1980) and Tombácz and Rice (1999) found that concentration could have an effect on the structure of organic compounds. With increasing concentration, the chance of intermolecular reactions is increased, resulting in a shift from lower molecular weight to higher

molecular weight. Luminescence spectra were obtained from an extract of air-dried muck. The undiluted samples showed strong peaks at wavelengths of 340 and 435, but when the extract was diluted those same peaks were not present in the spectra.

Whether or not the original attributes of humic substances are restored after dilution is unknown (Zsolnay, 2003). However, based on this current study and the IR spectra of the control blanks collected, concentration effects alter the structural make up and orientation of humic and fulvic acids' functionality after dilution. The primary chemical composition is not altered, but the results obtained after dilution do not necessarily project the function of the original organic molecule. This is a crucial point that is too often overshadowed in research. One should not assume that functional groups remain in the same orientation and the bonds of those functional groups making up the spectra, particularly shown in the fingerprint region, are the same during concentration then dilution. As stated previously, HS are complex and heterogeneous, meaning they are capable of re-orientation in order for binding to colloidal surfaces such as hydroxides or clay minerals.

Post adsorption FTIR spectra of FA and HA

FTIR spectral patterns of the FA after being exposed to the four mineral colloids are shown in Fig. 10-14 and the patterns for HA are shown in Fig. 15-19. Note that the patterns presented for both the FA and HA samples are the HS remnant material not adsorbed onto the colloid and the changes in spectral patterns were compared to the control acid spectra.

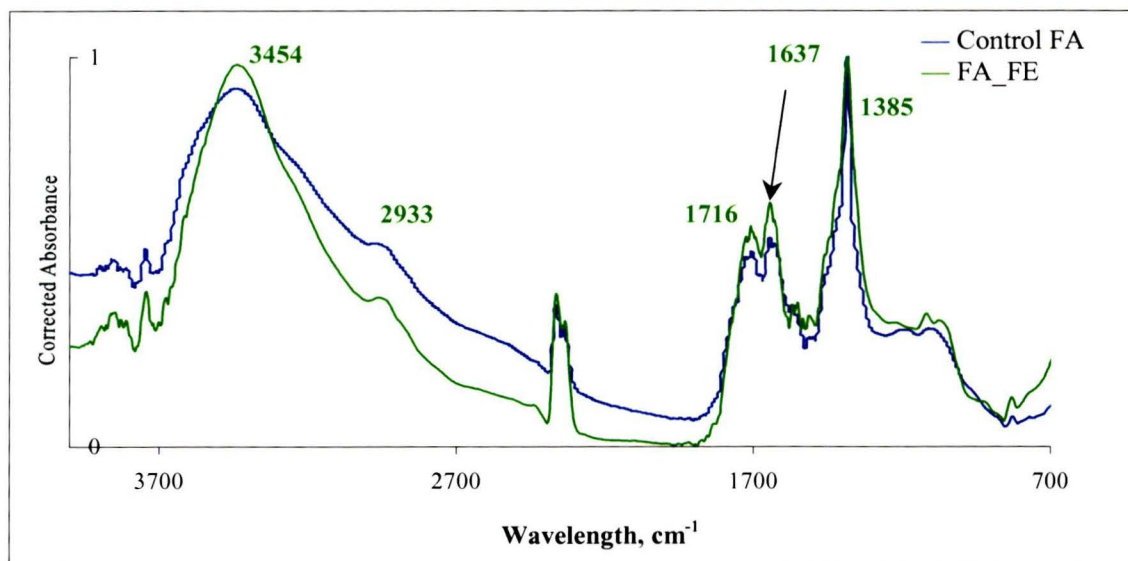


Fig. 10. FTIR spectra of FA after FE adsorption.

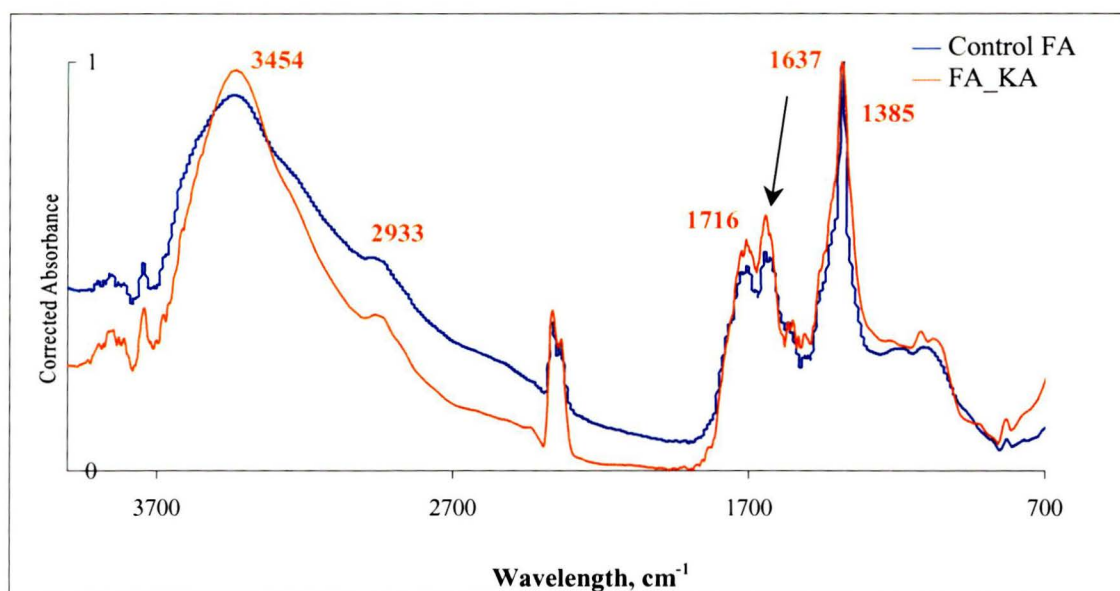


Fig. 11. FTIR spectra of FA after KA adsorption.

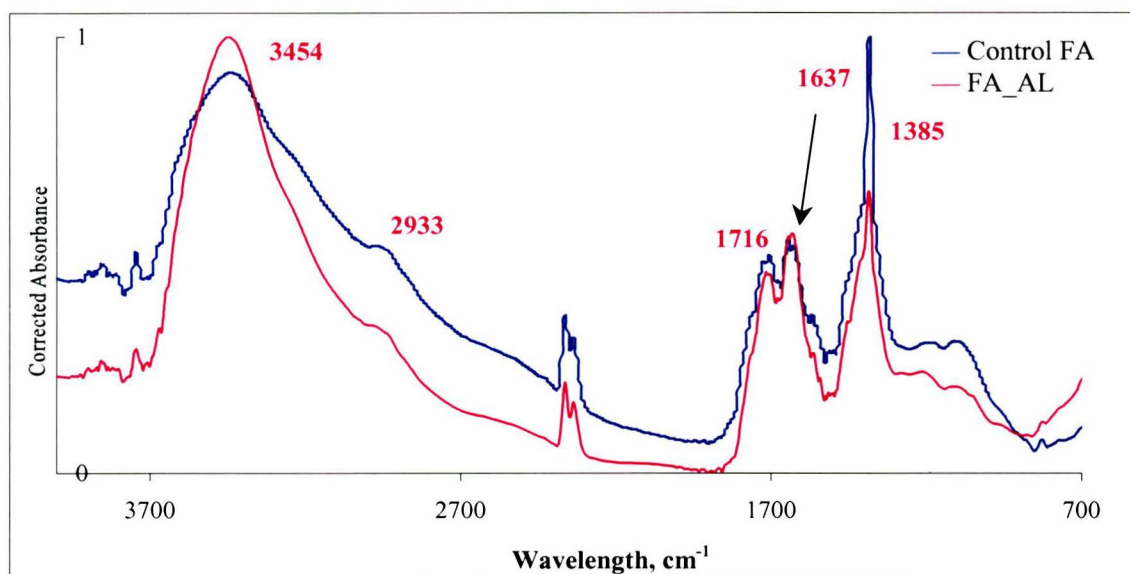


Fig. 12. FTIR spectra of FA after AL adsorption.

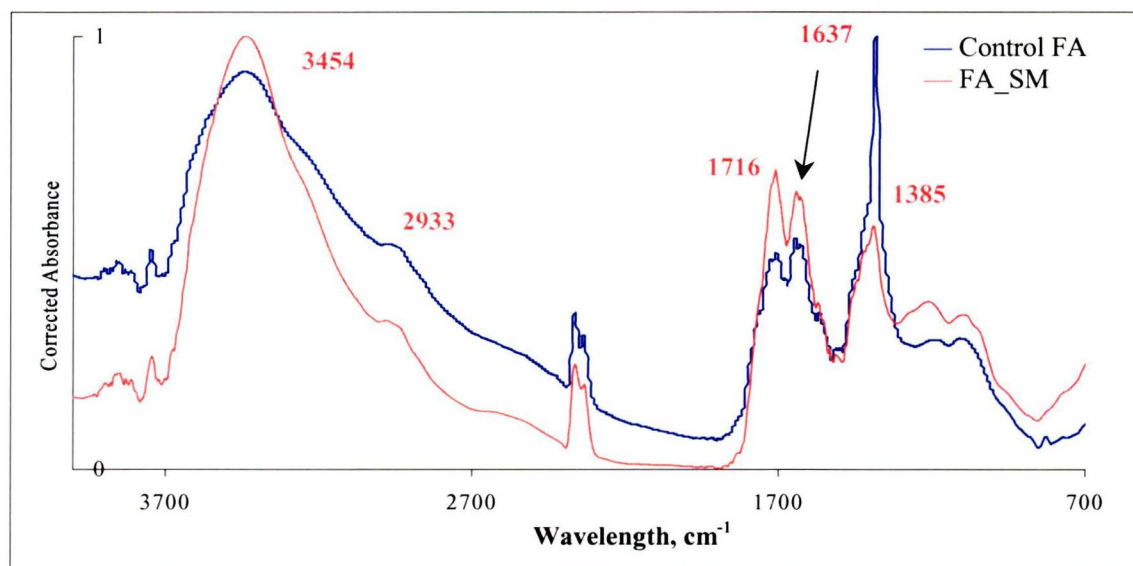


Fig. 13. FTIR spectra of FA after SM adsorption.

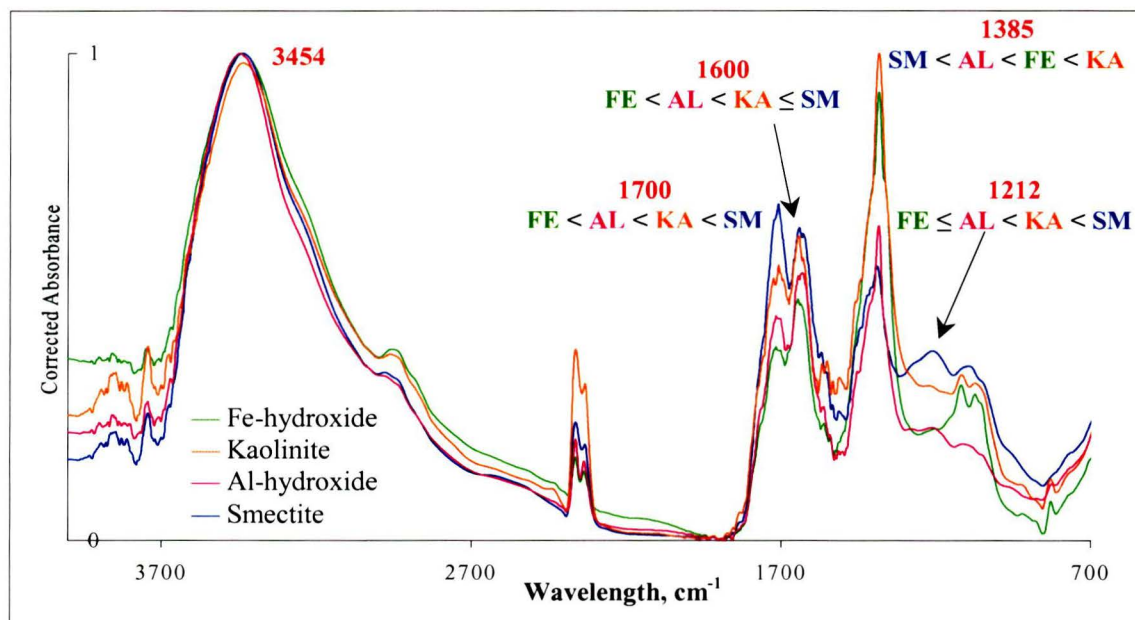


Fig. 14. FTIR spectra of FA after adsorption by the four colloids.

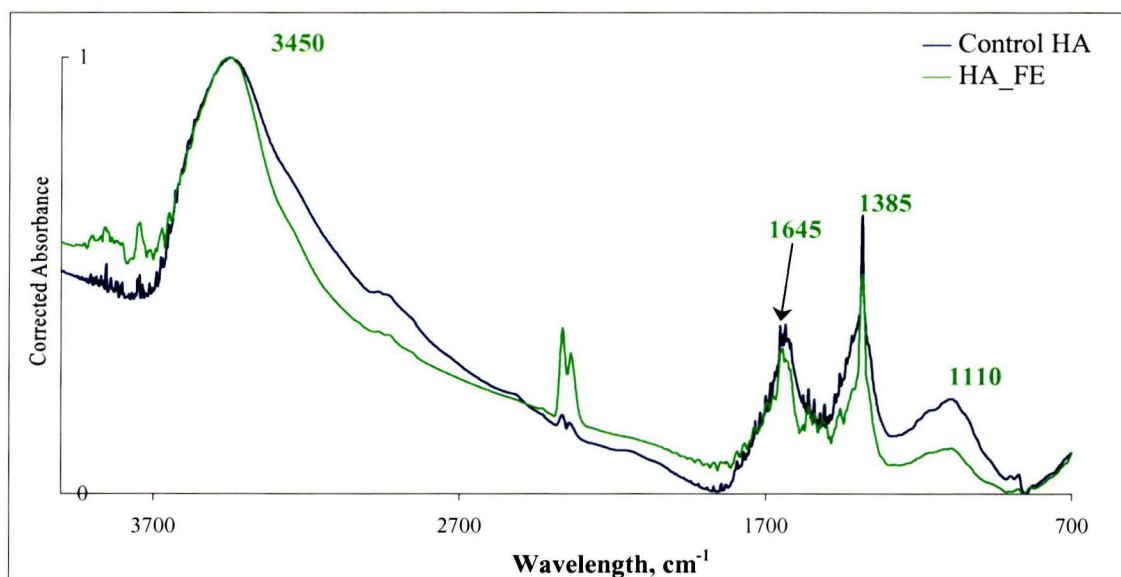


Fig. 15. FTIR spectra of HA after FE adsorption.

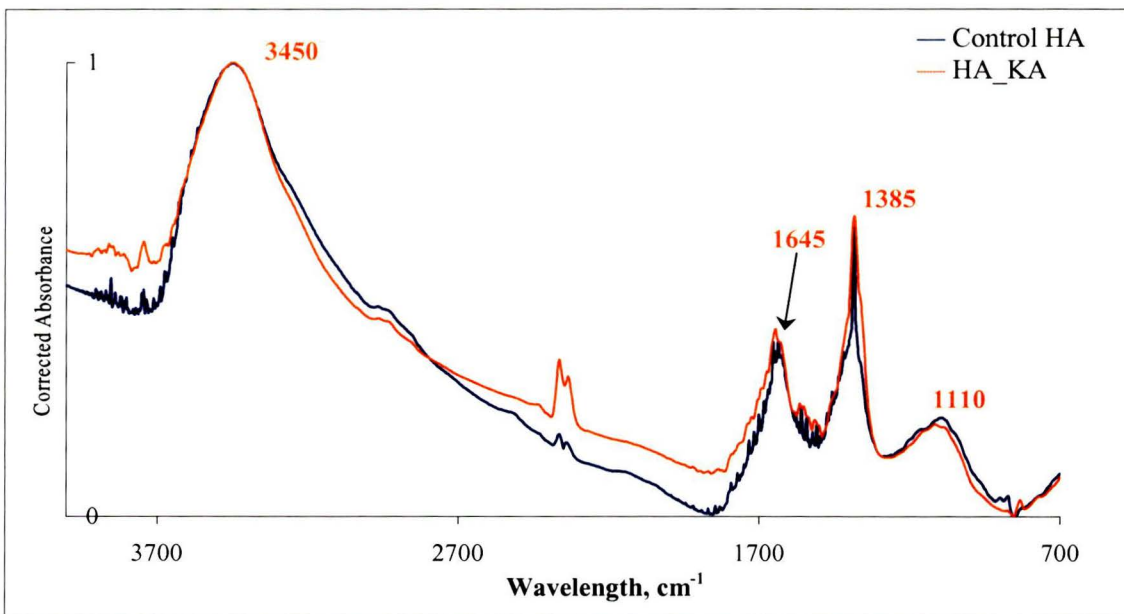


Fig. 16. FTIR spectra of HA after KA adsorption.

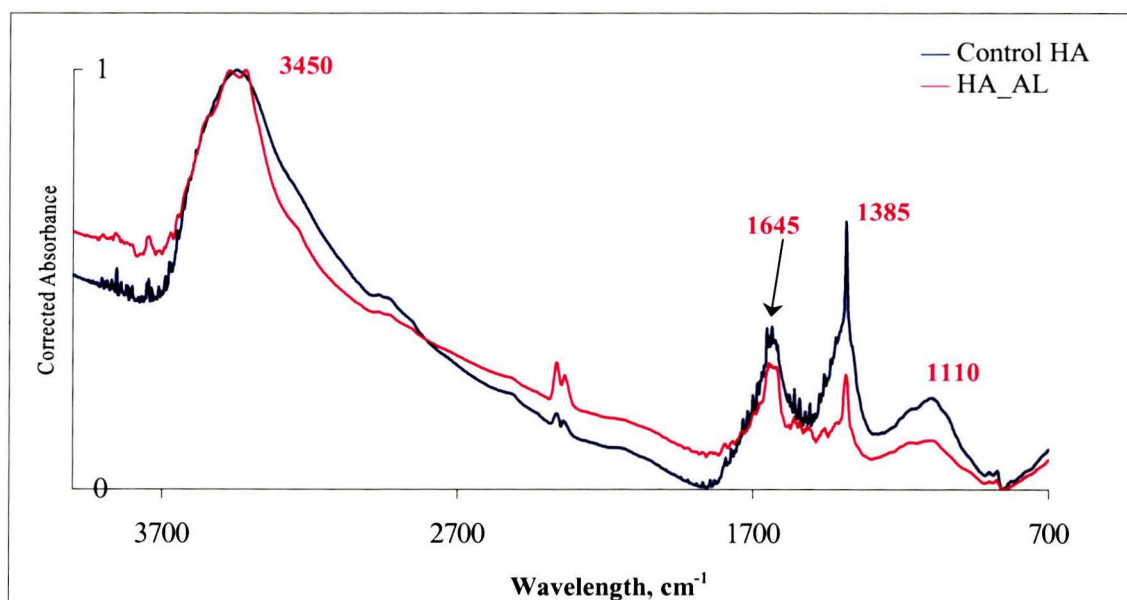


Fig. 17. FTIR spectra of HA after AL adsorption.

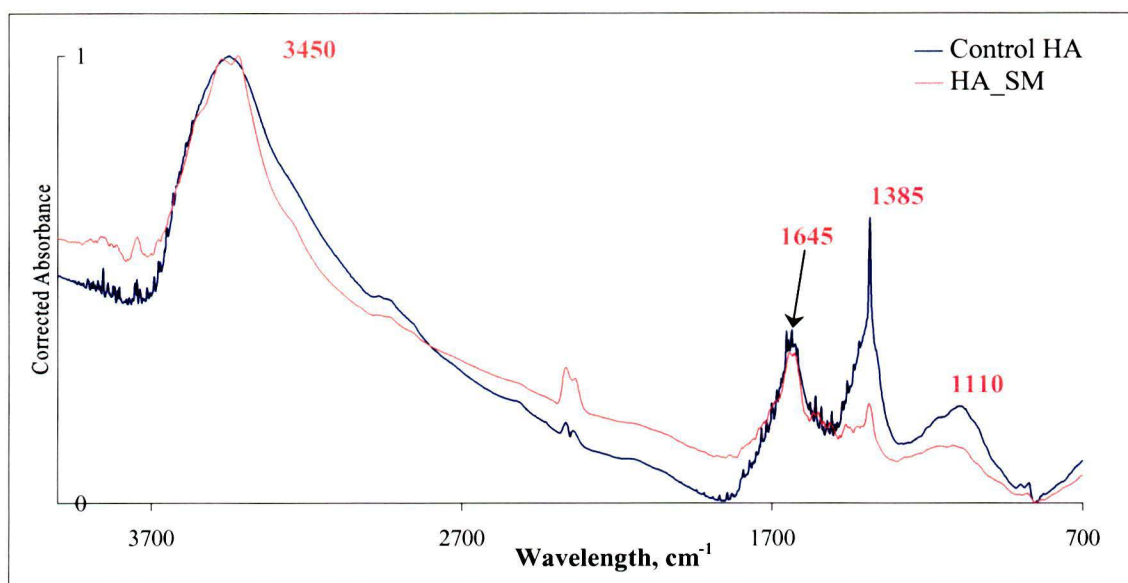


Fig. 18. FTIR spectra of HA after SM adsorption.

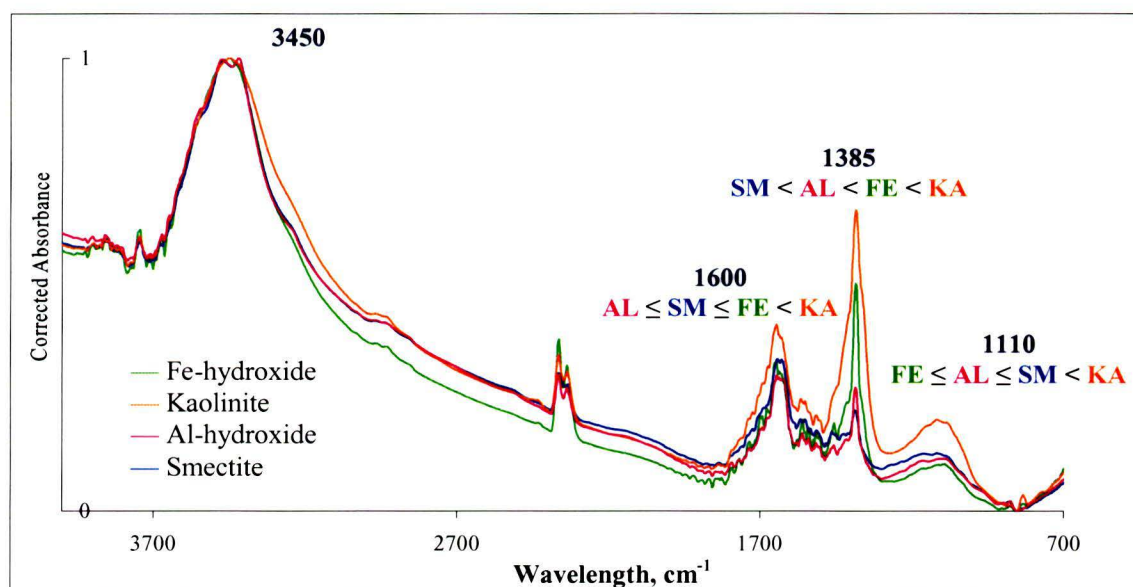


Fig. 19. FTIR spectra of HA after adsorption by the four colloids.

The 3454cm^{-1} (FA) and 3450cm^{-1} (HA) peaks after adsorption onto the four colloids showed a decrease in broadness, which explains that less H^+ bonding is occurring in the FA post adsorption within carboxylic group functionalities and further suggests that H^+ bonding may be more significantly involved in adsorption to mineral colloids contrary to other published literature. The 2933cm^{-1} peak of the FA became more pronounced after exposure to FE and KA, while the AL and SM are less sharp compared to its respective control pattern. This shoulder is associated with the aliphatic nature of the HS and may indicate that AL and SM adsorbed certain functional groups corresponding to bonds between C and H. The same shoulders on the HA samples for each colloid were less pronounced than the control samples.

Peak intensity sequences were made in order to identify the functional groups most likely involved in adsorption (Table 10). The 1700cm^{-1} peak of FA, which is associated with carboxyl groups decreased dramatically after FE adsorption and a slight decrease occurred from the AL. Studies have shown that FE sorbed carboxylic groups of NOM (Gu et al., 1995). Therefore, these observations correspond with other published

Table 10. Peak intensity sequences of FA and HA after adsorption exposure to the four colloids.

Peak ID	FA	HA
1700	FE < AL < KA < SM	N/A
1600	FE < AL < KA ≤ SM	AL < SM ≤ FE < KA
1385	SM < AL < FE < KA	SM < AL < FE < KA
1212	FE < AL < KA < SM	N/A
1110	N/A	FE < AL < SM < KA

data in that hydroxides sorb COOH groups. Conversely, the same FA peak increased in intensity from the KA and SM when compared to the control samples. This increase was probably due to removal of other functional groups, which may have been overwhelmed in the control samples. The 1600cm^{-1} peaks of FA increased in intensity for both SM and KA more than likely for the same reason. The intensity of the 1600cm^{-1} peaks for the HA samples remained relatively constant. However, the HA_AL sample decreased in intensity, due to AL surface hydroxyl site adsorption of COO^- .

Of all the peaks observed in the FA and HA samples, the 1385cm^{-1} showed the most variation between the colloids. This should not be surprising due to the fact that many organic functional groups are associated with this peak. In the characterization of the control FA and HA samples this peak was primarily identified as being associated with COO^- , OH deformation, and bonding of C-O from phenolic groups. The intensity decreased considerably for both the FA and HA samples after exposure to SM and AL, while it was generally unaffected after exposure to FE and KA. Therefore, the marked decrease may indicate that phenolic OH functional groups were adsorbed by these two colloids.

The 1212cm^{-1} peak of the FA sample when exposed to FE and AL decreased and increased after adsorption with SM. This peak, which was observed in the original HA samples disappeared in the control and colloidal exposure patterns. This region is associated primarily with carboxyl groups, which is probably why there was a decrease with FE and AL and an increase with SM.

Generally, the peaks associated with carboxyl groups showed a decrease in intensity in the FA and HA samples when exposed to FE and AL, suggesting the two

mineral hydroxides were selective in adsorption. The peaks corresponding to phenolic OH decreased in both the FA and HA samples after exposure to SM and AL. KA is the most difficult to assess in that no major changes occurred in the adsorption peaks only minor peaks arose, which could not be addressed.

Statistical Analysis of DOC data

ANOVA was performed on the DOC concentrations to examine the different existing combinations between acid and colloid and determine if interaction between these different combinations was significant. This analysis also aided in outlier detection. The METHODS chapter explains in detail how the analysis was performed. The execution of the statistical program can be found in APPENDIX F.

Acid, Colloid, and Acid*Colloid were three types of sources evaluated for significance at Type III SS error rate. The individual acid and colloid source had a $P < 0.0001$ value at 95% confidence, which indicates that these two sources are significant in the outcome of the DOC data. The Acid*Colloid source had a $P = 0.001$ at 95% confidence, also indicating this source was significant in the outcome of the DOC data. Once it was determined that interaction was occurring, Tukey Studentized Range Test was performed on the data set. This test created 8 different types of combinations between each acid and colloid. Each combination was then grouped, similar to cluster analysis, to determine if certain combinations were significantly or not significantly different from the others (Table 11).

Table 11. Results of Tukey Grouping of acid and colloid interaction. Fulvic acid (FA), Humic acid (HA), FE (iron-hydroxide), AL (aluminum hydroxide), KA (kaolinite), SM (smectite). Groups with the same letter are not significantly different.

Tukey Grouping		Interaction
	A	FA_SM
	A	FA_AL
	B	HA_SM
C	B	HA_AL
C	B	FA_KA
C		HA_FE
C		HA_KA
C		FA_FE

The results of the Tukey Test show interactions with the same letter are not significantly different and those with different letters are significantly different. The objectives of this study were to compare the similarities and differences existing between the four colloids in the adsorption of HA and FA.

The grouping analysis found the following combinations were not significantly different. Therefore, these combinations may show similarities in adsorption of HA or FA.

FA_FE and HA_KA	FA_SM and FA_AL
FA_FE and HA_FE	HA_AL and FA_KA.
HA_FE and HA_KA	

The grouping analysis also showed differences existing between the colloid and the acid used. The most observable variation occurs between the A and C groups. These groups divide the four colloids into two categories: (i) FA/HA_FE and FA/HA_KA and (ii) FA/HA_AL and FA/HA_SM. Therefore, differences exist not because of the acid, but the colloid. This suggests and validates the results from the experiment that the surfaces of the colloids contributed appreciably to the adsorption of humic and fulvic acids, as well as the mechanisms of that adsorption. The DOC data from the experiment, as well as the statistical analysis reveals that smectite adsorbed much differently than its clay counterpart, kaolinite. Additionally, the two hydroxides, which were originally expected to adsorb similarly are not grouped together.

Differences in adsorption were present between the two acids as well. From the Tukey grouping, the combinations between groups A and B were significantly different, which included the adsorption of HA versus FA onto aluminum hydroxide and smectite. Even though FE and KA adsorbed more HA than FA, the statistical analysis did not show that they were significantly different. Therefore, for adsorption by Fe-hydroxide and kaolinite, no significant differences in adsorption of HA or FA were apparent. However, for the adsorption by AL and SM, there were significant differences in adsorption of HA and FA.

Adsorption and Equilibrium Experiments

As explained in the Introduction, the specific (inner-sphere) adsorption behavior of organic matter compounds on oxide and clay surfaces is described by ligand-exchange reactions. The surface OH groups are exchanged by the aqueous anion. This mechanism

differs from non-specific (outer-sphere) adsorption because with non-specific adsorption, the adsorbing ligand is electrostatically held by a charged oxide or clay surface (Schulthess and MacCarthy, 1990). Both inner- and outer-sphere adsorption require anion exchange, and are, therefore, similar reactions. These types of adsorption mechanisms are competitive, but not independent of one another (Schulthess and Huang, 1991).

Adsorption and desorption of organic molecules is controlled by the chemical properties of the molecules and the surface properties of the colloid. The most relevant properties of the organic molecule are (i) identity of the functional groups attached (ii) acidity or basicity of the functional groups (iii) molecular size and shape (iv) polarity and charge of the molecule and (v) polarizability of the molecule. Controlling parameters for the colloids include pH, ionic strength and concentration (McBride, 1994).

The samples were analyzed for DOC concentration (mg C L^{-1}) to determine the amounts of HA or FA sorbed on each colloid. Table 12 and Fig. 20 show the average amounts of HA or FA adsorbed onto the four mineral colloids, which were calculated by subtracting the final DOC from the initial DOC concentrations then converting to mg HA or FA m^{-2} . The initial concentrations of the control HA and FA were 47.6 mg C L^{-1} and 31.2 mg C L^{-1} , respectively.

Table 12. Average amount of HA and FA adsorbed, mg HA or FA m^{-2} colloid.

	mg HA / m^2 colloid	mg FA / m^2 colloid
FE	0.3504	0.3343
KA	0.3503	0.2583
AL	0.1085	0.0287
SM	0.0890	0.0020

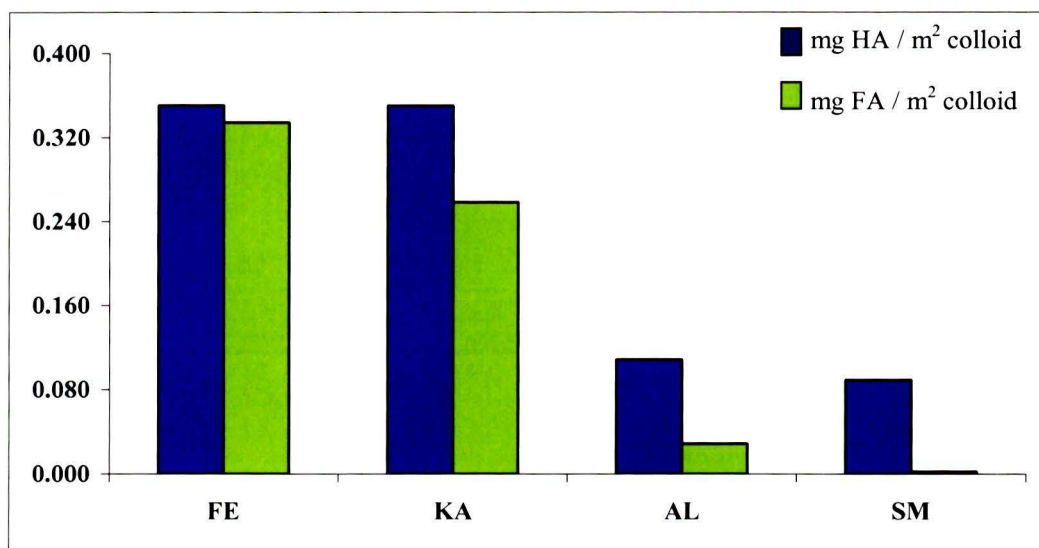


Fig. 20. Amounts of HA and FA adsorbed onto the four colloids at pH 4, mg HA or FA m⁻² colloid.

From the results two important observations were made, indicating (i) more HA was adsorbed by the four colloids than FA and (ii) Fe-hydroxide adsorbed more HA and FA than the other three colloids.

The major acid functional groups found in humic substances are carboxylic and phenolic groups. The carboxyl content of aquatic humic substances found in marshes, bogs and swamps have the lowest concentrations compared to rivers and streams, groundwater and seawater. HA is usually 20% less in carboxyl content than FA. Usually, HA contains 4.0 meq/g of carboxyl groups (one carboxyl group for every 12 carbon atoms), while aquatic FA contains 5.0 meq/g. Generally, carboxyl groups have an average pK_a around 4.

Humic acids have a slightly higher phenolic OH content (Malcolm, 1985), than does fulvic acid. The phenolic OH of humic substances has an average pK_a of 10. Therefore at the pH of the current study (4), the functional group is protonated. Since

ligand exchange is thought to be one of the mechanisms involved in adsorption of humic substances to hydroxide surfaces, HA may contain more exchangeable ligands (H₂O and OH) due to the nature of the HA at pH 4.

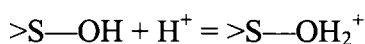
Strikingly, the amount of HA adsorbed compared to FA by SM and AL is considerable, which makes an obvious point that these two acids are quite different in their behavior, structure and composition for reasons known and unknown. SM sorbed approximately 0.089 mg HA m⁻² and 0.002 mg FA m⁻², while AL sorbed 0.109 mg HA m⁻² and 0.029 mg FA m⁻², approximately a 10x difference. The other two colloids adsorbed roughly double the amount of HA than FA (Table 12).

The observed sequence for both HA and FA in regards to the amounts of each organic acid sorbed onto the four colloids was as follows:

Fe-hydroxide > Kaolinite > Al-hydroxide > Smectite.

The sequence shows that more HA and FA was adsorbed by FE, than KA, AL and SM. This is in agreement with several studies that examined the adsorption behavior of goethite and its known specificity for humic substances (Meir et al., 1999).

The differences in adsorption between the two hydroxides are quite unexpected due to the similar nature of the reactive surface hydroxyl groups both contain. The results indicate that Fe-hydroxide adsorbed more HA and FA than Al-hydroxide. The shared charge of both the Al—OH and Fe—OH groups are the same. Both Fe and Al are trivalent cations and are coordinated with six oxygens giving a valence/coordination number of +0.5. At low pH the surface hydroxyl groups of these colloids (S) become protonated:



forming a positively charged surface after complexation. Hence, the distribution of charge should be the same for both Fe and Al hydroxides. The pH_{pzc} (point of zero charge) of the two hydroxides are relatively similar as well (McBride, 1994).

Schnitzer and Khan (1980) reported on the adsorption of humic acid and its affinity for Al or Fe metals. They found that Fe tended to out compete Al. Gu et al. (1994) also states that the carboxyl and hydroxyl functional groups of humic and fulvic acids are known to form very stable complexes with many metal cations or hydroxyl metal cations, which follows the sequence: $\text{Fe}^{3+} > \text{Al}^{3+} > \text{Pb}^{2+} > \text{Ca}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+}$.

This current study found that FE had a higher affinity for a FA than kaolinite, which also agrees with the findings of Namjesnik-Dejanovic et al. (2000) and Mier et al. (1999) (See CHAPTER I, pg. 15). In addition, Al-hydroxide, which has an abundance of active Al-OH sites adsorbed less HA and FA compared to kaolinite. This is due to isomorphous substitution of some Fe^{3+} for Al^{3+} in the octahedral layers of kaolinite. Therefore, the results of the current study are consistent with reported data, in which Fe-hydroxide adsorbed more than the two clay minerals.

Smectite adsorbed less HA and FA than the other colloids. As typical characteristics, smectite groups are known for their 2:1 layered structure and permanent negative charge. These clays are comprised of two tetrahedral sheets and one octahedral sheet. Within these layers, Mg^{2+} is usually substituted for Al^{3+} and less commonly, Al^{3+} substituted for Si^{4+} . Nevertheless, both types of substitution cause the development of negative permanent charge (McBride, 1994; Brady and Weil, 2002). Thus, the net permanent negative charge on the smectite clay micelle and the negative charges of both the HA and FA at pH 4 induced electrostatic repulsive forces contributing to less

adsorption than the other three colloids. Interestingly, the colloids that maintained a positive pH-dependent charge, at the experimental conditions, adsorbed the most HA and FA compared to smectite, which has very few pH dependent charge sites. Isomorphous substitutions within the octahedral sheets between Mg^{2+} and Al^{3+} and the loss of Al^{3+} centered sites decreased the amounts of HA and FA adsorption. As reported previously, Al centered sites are preferred by humic substances over Mg sites (Gu et al., 1994).

The structural arrangement of the smectite creates two external silica tetrahedral surfaces and one internal octahedral surface. Because of their “internal” position, the aluminum sites (not isomorphically substituted) of the octahedral layer tend to be isolated causing adsorption to be hindered. The results from the current study indicated that SM adsorbed more HA than FA. Schulthess and Huang (1991) studied the adsorption between HA and FA and mordenite (a zeolite). This study found that the % FA removed at pH 4 was much less compared to the % HA removed and suggested that the adsorption by zeolite is dominated by Si sites. They attribute the differences in adsorption due to the pore-channel selectivity system of zeolites. That is, the % FA adsorbed was less than the % HA adsorbed due to size exclusion phenomena; this assumes the lack of Al sites on the external zeolite surface. Therefore, there could be a higher degree of selectivity of HA for Si sites at this pH accounting for the higher adsorption that occurred with HA.

The differences between adsorption of HA and FA by the four colloids reflect the differences in adsorption mechanisms—ligand exchange, hydrogen bonding, anion exchange etc. FA may contain a higher density of functional groups that form multiple adsorption bonds at a certain pH, while HA may contain another set of functional groups that form multiple bonds (Schulthess and Huang, 1991). At pH 4, FE, AL, and KA had

the most positive charges and possibly a more uniform set of surface hydroxyls hence more sorption of the negatively charged humic substances occurred.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Humic and Fulvic acids are complex macromolecular substances containing free and bound functional groups including phenolic OH and carboxylic acids. Their structure is unknown and spectroscopy is being used in order to gain and understanding of their characterization. Fourier Transform Infrared (FTIR) was the spectroscopic technique used in identifying these functional groups for the current study.

The spectral patterns of the humic and fulvic acids from the Dismal Swamp showed no significant variation in the functional groups they contain except at 1034cm^{-1} and 913cm^{-1} HA, only differences of absorption peak intensity were observed. Higher amounts of carboxyl groups were seen in the FA sample. This indicates that some functional groups may be the same, but the actual framework between the two is different. Therefore, more improved spectroscopic methods or techniques used in conjunction with FTIR need to be employed to understand the “real” differences of HA and FA, instead of the common operational definitions they have been assigned.

An important issue raised in this current study are these operational definitions, which are related to the techniques used to isolate these two fractions. Most of the techniques currently in practice are concentration methods to collect enough material from aquatic systems for laboratory study. The concentration effects on the HA and FA structure have not been examined. This current project found that the spectra of HA and FA changed dramatically upon dilution. More research is needed on the concentration and dilution effects on the structure of HA and FA.

The mechanisms by which HA and FA are adsorbed to mineral colloids were shown to be different. HA and FA contain variable orientations and amounts of functional groups, which caused different amounts to be sorbed to the different colloids. More HA was sorbed by the four colloids compared to FA. FE adsorbed more HA and FA than the other three colloids, which is in agreement with other published data. Therefore, FE seemed to be the preferred colloid for adsorption. This should not be surprising due to its high surface-OH exchange groups.

Results indicated that FE and KA adsorbed similarly and AL and SM likewise. Therefore, the two hydroxides that were expected to adsorb similarly did not. The surfaces of the two hydroxides were similar in nature at pH 4 in respect to their surface chemistry. Thus, there is a degree of selectivity shown by the HA and FA for the hydroxide species, which follows a similar pattern to ion selectivity ($\text{Fe}^{3+} > \text{Al}^{3+}$).

Smectite adsorbed the least HA and FA of all four colloids. This clay mineral group is known for its 2:1 structure capable of expansion and exchange. However, the net (permanent) negative charge and little pH dependent sites, induced electrostatic repulsive forces of both HA and FA contributing to less adsorption.

The implications of the results from the current study indicate that in natural systems, the adsorption of aqueous compounds is extremely dependent on the types of surfaces present in the outermost layer of the solid phase and also the composition and structure of those surfaces. The ability to predict the adsorption behavior of humic substances requires a method that can determine the amounts of exposed surface reactive sites (adsorption sites) as well as the relative amounts of ligands available for exchange within HA and FA.

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APPENDIX A

PROCEDURES FOR

COLLOIDAL SYNTHESIS

PROCEDURE FOR PREPARING $\text{Fe}(\text{OH})_3$ PRECIPITATE

Schwertmann and Cornell, 1991

- 1) Pour 100 mL of $\text{Fe}(\text{NO}_3)_3$ solution (freshly prepared by dissolving unhydrolyzed $\text{Fe}(\text{NO}_3)_3 - 9\text{H}_2\text{O}$ in H_2O) in a 2 L polyethylene flask.
- 2) Rapidly add 180 mL of 5 M KOH.
- 3) A red-brown ferrihydrite precipitates at once.
- 4) Immediately dilute the suspension to 2 L with DI H_2O .
- 5) Hold in a closed polyethylene flask at 70°C for 60 hours. During this period the red brown suspension is converted to a yellow brown precipitate of goethite.
- 6) Remove the flask from the oven.
- 7) Centrifuge.
- 8) Wash and freeze-dry the precipitate.

PROCEDURE FOR PREPARING $\text{Al}(\text{OH})_3$ PRECIPITATE

Kinniburgh et al., 1975

- 1) Pour 100mL of 1 M $\text{Al}(\text{NO}_3)_3$ solution (freshly prepared by dissolving unhydrolyzed $\text{Al}(\text{NO}_3)_3 - 9\text{H}_2\text{O}$) in a 2L polyethylene flask/beaker.
- 2) Add 5 M KOH to the solution until a pH 6.5 is obtained.
- 3) Immediately dilute the suspension to 2L with DI H_2O .
- 4) A white precipitate of aluminum hydroxide forms.
- 5) Hold in a closed polyethylene flask at 70°C for 60 hours.
- 6) Remove the flask from the oven.
- 7) Centrifuge.
- 8) Wash and freeze-dry the precipitate.

**PROCEDURE FOR CLAY MINERAL SEPARATION BY
SEDIMENTATION: < 2 μ m SIZE FRACTION**
Jackson, 1969

- 1) Grind the clay material in a mortar and pestle until all aggregates are broken and a fine powder is obtained. The final suspension concentrations for settling should be no more than 2%, so adjust the sample in accordance with the volume of the settling container.
- 2) Place the material into one or more plastic bottles and add dilute Na-hexametaphosphate and agitate on an end-to-end shaker for 10-20 minutes.
- 3) Remove the bottles from the shaker and allow them to sit undisturbed for 5 minutes (not over 10 minutes!) to allow the coarse silt to settle.
- 4) Decant the suspension into a 1 L (or larger) beaker making sure that the coarser material in the bottom of the bottles is not transferred.
- 5) Add more of the Na-hexametaphosphate solution to the bottle and repeat the process until the clay and fine silt are separated from the coarser material. This may take up to 5 cycles.
- 6) Add the clay/fine silt suspension to a large beaker or jug and fill to desired height with DI H₂O. Stir the suspension and calculate the settling time for 2 μ m clay particles for a convenient time. Refer to pp. 114 and 115 in Jackson, 1969 to calculate proper settling times, noting the lab temperature.
- 7) At the end of the settling period, use glass tubing with an upward curve at the bottom (to prevent removal of coarser material below the settling zone). Siphon the < 2 μ m into a beaker or jug with a capacity larger than the sedimentation container.
- 8) Refill the jug with the coarser material with the Na-hexametaphosphate solution, re-stir, let settle and remove the clay as before.
- 9) To the < 2 μ m clay suspension add a few drops of 1 M MgCl₂ and mix. The clay particles should flocculate within a short time. If flocculation does not occur, add more of the MgCl₂.
- 10) After the clay has settled, remove the clear liquid and discard. Continue to accumulate the < 2 μ m clay into this container until the original sample is void of this sized material.
- 11) If needed/desired regrind the coarse material from Steps 3 and 4 and repeat the entire separation process.

- 12) After all of the $< 2\mu\text{m}$ material has been collected, reduce the volume via centrifugation using 250 mL bottles and rinsing with DI H_2O to remove all excess salts. If required, re-saturate with the desired cation (e.g. Na^+) at this time. Transfer the suspensions to one container, mix well to obtain the stock clay suspension.
- 13) Freeze-dry to obtain solid form.

APPENDIX B

PROCEDURE FOR DETERMINING

COLLOIDAL SURFACE AREA

PROCEDURE FOR DETERMINING COLLOIDAL SURFACE AREA

Carter et al., 1986

Preparation of CaCl_2 —EGME solvate

- 1) Weigh approximately 120 g of 40-mesh CaCl_2 into a 1 L beaker.
- 2) Dry in an oven at 210°C for 1 h or more to remove all traces of water.
- 3) Weigh 20 g of EGME into a 400-mL beaker.
- 4) Remove the CaCl_2 from the oven and weigh out 100 g without cooling, add to the beaker containing the EGME.
- 5) Mix immediately and thoroughly with a spatula.
- 6) The heat of the CaCl_2 facilitates solvation.
- 7) After the solvation has cooled, transfer it to a culture chamber and spread it uniformly over the bottom.
- 8) Store the chamber and contents in a sealed desiccator.

Sorption of EGME technique

- 1) Weigh approximately 1.1 g of material (clay or oxide) into a tared aluminum can, including a lid and spread the sample evenly over the bottom of the can.
- 2) Place the can, with lid beneath, in a vacuum desiccator over about 250 g of P_2O_5 .
- 3) Evacuate the desiccator by applying a vacuum pump for one hour, close the stopcock and dry to constant weight.
- 4) Constant weight is usually reached in about 6 to 7 h for groups of four to six samples.
- 5) Weigh the dried sample, using care to minimize adsorption of atmospheric water.
- 6) Wet the sample with approximately 3 mL of reagent-grade ethylene glycol monoethyl ether (EGME) to form a material-adsorbate slurry.
- 7) Place the can containing the sample-adsorbate slurry, with lid beneath, in a culture chamber on the hardware cloth support over the CaCl_2 -EGME solvate.

- 8) Place the lid on the culture chamber (elevation of the lid with a small block to leave a space approximately 2 mm wide between lid and chamber will better allow gases to escape).
- 9) Place the entire culture chamber in a vacuum desiccator containing CaCl_2 .
- 10) Allow 30 min or more for the sample-solvate slurry to equilibrate.
- 11) Evacuate the desiccator with a vacuum pump for about 45 min.
- 12) Allow the desiccator to stand at room temp for 4 to 6 h, release the vacuum, open the desiccator and culture chamber, and place the lid on the aluminum can to prevent the sample from adsorbing atmospheric water.
- 13) Weigh the can, lid and sample.
- 14) Return the can, with lid beneath, to the culture chamber and the culture chamber to the desiccator.
- 15) Evacuate the desiccator by applying a vacuum pump for 45 min.
- 16) Weigh the samples at 2- to 4-h intervals, evacuating between weighings, until constant weight is attained (takes 2 or 3 weighings).
- 17) Use the mean of the two successive weights that agree within a few tenths of a milligram to calculate the quantity of EGME retained by the sample.

Calculating Surface Area

Calculate the specific surface by the equation:

$$A = W_a / (W_s \times 0.000286)$$

where A = specific surface in m^2/g , W_a = weight of EGME retained by the sample in g, W_s = weight of P_2O_5 -dried sample in g and 0.000286 is the weight of EGME required to form a monomolecular layer on a square meter of surface.

APPENDIX C

PROCEDURE FOR RESIN CLEANING

AND GLASS COLUMN PACKING

PROCEDURE FOR RESIN CLEANING AND GLASS COLUMN PACKING

Thurman and Malcolm, 1981

- 1) Initially remove all fines while in DI H₂O.
- 2) Clean by washing the beads in 0.1N NaOH and then rinsing the resin with DI H₂O water.
- 3) Next, rinse the resin with methanol and acetonitrile. Repeat this process twice. Store the clean resin in methanol until used.
- 4) The glass columns should be packed with an H₂O-resin slurry and rinsed with DI H₂O water to remove methanol:
 - a) Add approximately 1" of DI H₂O to the empty column before adding the resin slurry.
 - b) Slowly pour the resin slurry into the column.
 - c) As the column fills, drain excess water through the bottom of the column, but do not allow the liquid level to fall below the top of the resin bed.
 - d) Add enough resin to half-fill the column only. This will allow room for expansion when resin-swelling solvents are used.
- 5) Rinse the packed glass column 3 times alternating from 0.1N NaOH to 0.1N HCl immediately before using. This removes impurities, which may otherwise be incorporated into the sample.
- 6) This rinse must precede any sample application.

APPENDIX D

PREPARATION OF HUMIC AND

FULVIC ACID SEPARATION

PREPARATION OF HUMIC AND FULVIC SEPARATION

Thurman and Malcolm, 1981

- 1) Pre-filter water samples using a 1.2 μ m Gelman type A/E glass fiber filter followed by a 0.4 μ m polycarbonate membrane filter.
- 2) Acidify the filtered water to pH 2.
- 3) Pass sample through 245mL (2.5 x 10 cm) DAX-8 column using a peristaltic pump and Teflon tubing at 15 bed volumes per hour to retain HA and FA.
- 4) In reverse direction, elute the HA and FA with 3 void volumes of 0.1 N NaOH from the column at a flow rate of 5 bed volumes per hour.
- 5) Acidify IMMEDIATELY with HCl to pH 2 to avoid oxidation of humic substances.
- 6) Re-concentrate the eluate on a smaller 98mL (1.5 x 10 cm) DAX-8 column.
- 7) Elute with 0.1 N NaOH (step 4).
- 8) Adjust eluate to pH 1 with HCl to precipitate humic acid for 24h.
- 9) Separate the humic and fulvic acids by centrifugation. Collect HA on a 0.025 μ m membrane filter and wash with dilute HCl to remove ash.
- 10) Rinse humic acid fraction with distilled water until AgNO₃ test shows no Cl⁻ in wash water.
- 11) Dissolve HA in 0.1 N NaOH and hydrogen saturate by passing solution through cation-exchange resin in H-form.
- 12) Reapply FA at pH 2 on smaller DAX-8 column.
- 13) Desalt FA by rinsing column with 1-void volume of distilled water to remove HCl and inorganic salts.
- 14) Elute FA by back-elution with 0.1 N NaOH.
- 15) IMMEDIATELY pass the 0.1 N NaOH eluate through cation-exchange resin in H⁺-form. Continue cation-exchange process until final concentration of Na⁺ is less than 0.1 ppm.
- 16) Freeze-dry HA and FA fractions.

APPENDIX E
pH MEASUREMENTS OF FILTRATE
AFTER ADSORPTION

Table A1. pH Measurements of Filtrate After Adsorption

HADI R1	4.35
HADI R2	4.00
HADI R3	4.20
FADI R1	4.66
FADI R2	4.75
FADI R3	5.01
HAFE R1	4.12
HAFE R2	3.71
HAFE R3	3.99
FAFE R1	6.18
FAFE R2	4.16
FAFE R3	3.93
FEDI R1	4.00
FEDI R2	3.83
FEDI R3	4.30
HASM R1	4.03
HASM R2	4.34
HASM R3	4.14
FASM R1	4.21
FASM R2	4.31
FASM R3	4.02
SMDI R1	3.89
SMDI R2	5.91
SMDI R3	5.62
HAAL R1	4.19
HAAL R2	4.09
HAAL R3	3.96
FAAL R1	4.36
FAAL R2	4.45
FAAL R3	4.36
ALDI R1	4.83
ALDI R2	4.80
ALDI R3	5.29
HAKA R1	4.07
HAKAR2	4.19
HAKA R3	4.16
FAKA R1	4.00
FAKA R2	3.65
FAKA R3	3.20
KADI R1	4.41
KADI R2	4.59
KADI R3	3.83

APPENDIX F
ANOVA MULTIVARIATE TESTS AND
TUKEY GROUPING
OF DOC DATA POST ADSORPTION

Table A2. ANOVA Multivariate Tests and Tukey Grouping of DOC Data Post Adsorption

The SAS System						1
11:37 Friday, October 10, 2003						
The GLM Procedure						
Class Level Information						
Class	Levels	Values				
Acid	2	Fa Ha				
Mineral	4	Al Fe Ka Sm				
Number of observations						22
The SAS System						2
11:37 Friday, October 10, 2003						
The GLM Procedure						
Dependent Variable: Y						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	7	1898.344001	271.192000	43.96	<.0001	
Error	14	86.360728	6.168623			
Corrected Total	21	1984.704729				
R-Square	Coeff Var	Root MSE	Y Mean			
0.956487	14.69660	2.483671	16.89964			
Source	DF	Type III SS	Mean Square	F Value	Pr > F	
Acid	1	353.102403	353.102403	57.24	<.0001	
Mineral	3	1280.066244	426.688748	69.17	<.0001	
Acid*Mineral	3	181.625387	60.541796	9.81	0.0010	
The SAS System						3
11:37 Friday, October 10, 2003						
The GLM Procedure						
Class Level Information						
Class	Levels	Values				
Acid	2	Fa Ha				
Mineral	4	Al Fe Ka Sm				

Number of observations 22

The SAS System

4

11:37 Friday, October 10, 2003

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1898.344001	271.192000	43.96	<.0001
Error	14	86.360728	6.168623		
Corrected Total	21	1984.704729			

R-Square	Coeff Var	Root MSE	Y Mean
0.956487	14.69660	2.483671	16.89964

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Acid	1	353.102403	353.102403	57.24	<.0001
Mineral	3	1280.066244	426.688748	69.17	<.0001
Acid*Mineral	3	181.625387	60.541796	9.81	0.0010

The SAS System

5

11:37 Friday, October 10, 2003

The GLM Procedure

Class Level Information

Class	Levels	Values
Int	8	Fa_A1 Fa_Fe Fa_Ka Fa_Sm Ha_A1 Ha_Fe Ha_Ka Ha_Sm

Number of observations 22

The SAS System

6

11:37 Friday, October 10, 2003

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1898.344001	271.192000	43.96	<.0001

Error	14	86.360728	6.168623
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Corrected Total	21	1984.704729
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R-Square	Coeff Var	Root MSE	Y Mean
0.956487	14.69660	2.483671	16.89964

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Int	7	1898.344001	271.192000	43.96	<.0001

The SAS System 8
11:37 Friday, October 10, 2003

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Y

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	14
Error Mean Square	6.168623
Critical Value of Studentized Range	4.99029
Minimum Significant Difference	7.5899
Harmonic Mean of Cell Sizes	2.666667

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Int
A	33.559	3	Fa_Sm
A			
A	27.441	3	Fa_A1
B	19.068	3	Ha_Sm
B			
C B	14.207	3	Ha_A1
C B			
C B	12.509	3	Fa_Ka
C			
C	7.559	2	Ha_Fe
C			
C	7.316	3	Ha_Ka
C			
C	7.188	2	Fa_Fe

VITA

Sarah Elizabeth Diebel received a B.S. in Marine Science from Coastal Carolina University, Conway, SC in May 2000. In August 2000, Sarah began working toward a Master of Science degree in Geology at Old Dominion University, Norfolk, VA. Sarah worked as a Graduate Teaching Assistant for two years (8/00-5/02) responsible for classroom laboratory lecture, exercises and grading. Sarah then worked her last year as a Graduate Research Assistant (5/02-12/03). During this time she completed her thesis research. Sarah is currently working at the Natural Resources Conservation Service for the USDA in Chesapeake, VA. Sarah received her M.S. in Geology from the Ocean, Earth and Atmospheric Sciences, Old Dominion University 4600 Elkhorn Avenue Norfolk, Virginia 23529 in December 2003.